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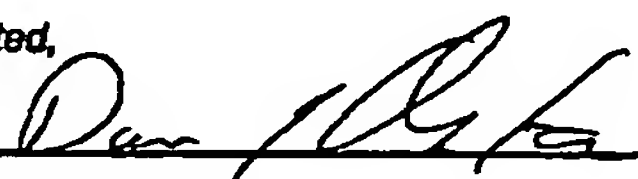
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INVENTOR(S)					
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)			
Heike Inge Ada	Winter	Raleigh, NC, US			
Steven C	Huber	Savoy, IL, US			
Carolyn A	Larabell	Oakland, CA, US			
Additional inventors are being named on the _____ separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
Synthetic Peptides that Cause F-Actin Bundling and Block Actin Depolymerization					
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<input type="checkbox"/> No.					
<input checked="" type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: <u>US Dept of Energy</u> <u>DE-AC03-76SF00098, DE-AI01-91ER20031, DE-AI05-91ER20031</u>					

[Page 1 of 1]

Respectfully submitted,

SIGNATURE

TYPED or PRINTED NAME David J AstonTELEPHONE 510-495-2839Date 10/20/03REGISTRATION NO. 28,051

(If appropriate)

Docket Number: JIB-1571P**USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT**

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# **SYNTHETIC PEPTIDES THAT CAUSE F-ACTIN BUNDLING AND BLOCK ACTIN DEPOLYMERIZATION**

Inventors: Heike Winter, Steven Huber and Carolyn Larabell

## **STATEMENT OF GOVERNMENTAL SUPPORT**

[001] This invention was made during work supported by the U.S. Department of Energy under Contract Nos. DE-AC03-76SF00098 and DE-AI01-91ER20031 and the USDA-ARS under Contract No. DE-AI05-91ER20031. The government has certain rights in this invention.

## **BACKGROUND OF THE INVENTION**

### **FIELD OF THE INVENTION**

[002] This invention generally relates to the field of chemotherapeutic agents useful in the inhibition of cytokinesis and cell division for the research and treatment of cancers and other diseases.

## **DESCRIPTION OF THE RELATED ART**

[003] Highly regulated assembly and disassembly of actin monomers into filaments and bundles ("F-actin") is the essential basis for cell shape, cytokinesis and motility in eukaryotic cells. Actin filaments build a dynamic intracellular structure in all eukaryotic cells. These filaments as part of the cytoskeleton stabilize the cell and provide a network for unidirectional movement of proteins, which can mediate the localization of other proteins, mRNA or entire organelles. Actin binding proteins are involved in the organization of the actin filament network itself by crosslinking, capping or anchoring these microfilaments to membranes. The most abundant actin binding protein in a cell is actin itself. The actin monomer (ca. 43 kD) has four actin binding sites, which enables it to polymerize into filaments of different size and organization.

[004] Polymerization of actin requires binding of ATP and subsequent hydrolysis into ADP and Pi. This exergonic reaction induces a conformational change in the monomer, exposing

actin:actin binding sites. Only a few substances are known to modify the polymerization of actin. These compounds are useful tools to study the coordination and functions of the actin cytoskeleton in the cell.

[005] Inhibiting cytoskeletal dynamics is one of the most powerful strategies employed in cancer treatment. Examples of compounds that bind to actin and inhibit cytoskeletal dynamics include such commonly used cancer drugs such as paclitaxel, eleutherobin, epithilone and discodermalide. However, drugs commonly used for this purpose cause serious side effects on fast-growing cells such as bone marrow cells, hair cells, intestinal brush border cells and germinal cells, as degradation of these chemicals is slow. This problem is compounded by cumulative cytotoxicity on peripheral organs.

[006] The present invention relates to peptides derived by the inventors from sucrose synthase. Sucrose synthase (SuSy) is recognized as an important enzyme of sucrose (Suc) utilization in plant sink tissues. (L.C. Ho., *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 39 (1988), pp. 355-378). In particular, the highest activity of SuSy often occurs during rapid growth (e.g. elongating maize leaves as described by B. Nguyen-Quoc, et al., *Plant Physiol.* 94 (1990), pp. 516-523) or during storage product deposition (e.g. developing seeds, as shown by S.S. Sung, et al., *Plant Cell Environ.* 17 (1994), pp. 419-426). SuSy is a globular protein and thus is generally considered to be soluble in the cytosol. However, some of the enzyme is known to be associated with the plasma membrane, perhaps in a specific complex with glucan synthase(s) in the membrane. Evidence that soluble SuSy binds to both G- and F-actin *in vitro*, as well as evidence that some of the SuSy may be associated with actin *in situ* is described in H. Winter, et al., *FEBS Letters*, Volume 430, Issue 3, 3 July 1998, pages 205-208.

[007] An alternative approach to the use of complex organic molecules to inhibit cell proliferation is the use of synthetic peptides. While other peptides have been shown to cause F-actin bundling *in vitro*, those peptides are generally highly basic in composition and promote formation of lateral aggregates of F-actin in a rather non-specific manner. In the long run, these compounds offer the prospect of minimalizing side effects by tumor cell-directed transfection of

the sequences encoding the drug; in the short run, as peptides are inherently more easily degradable by the organism, they are not expected to cause cumulative cytotoxicity.

### BRIEF SUMMARY OF THE INVENTION

[008] The present invention comprises peptides loosely derived from the sequence of the *Zea mays* enzyme, Sucrose Synthase (SuSy), at amino acids 375-389 (Gen Bank Accession No. P49036). The present peptides share homology with many other sucrose synthases of other plant origin. The application of the active peptides of this invention results in stabilization of microfilaments and causes cross-linking of F-actin *in vitro* and *in situ*. The peptides of the present invention, along with inactive control peptides, may be characterized as follows, where the conventional single letter amino acid code letters are used and the derivative source of the peptide is indicated:

SEQ ID NO: 1	ENGIVRKWISRFEVW	Consensus active peptide SuSy 1
SEQ ID NO: 2	ENGILRKWISRFDVW	<i>Zea mays</i> SuSy1 367-381
SEQ ID NO: 3	ENGIVRKWISRFEVW	<i>Zea mays</i> SuSy2 375-389
SEQ ID NO: 4	ENGILKKWISRFDVW	<i>Zea mays</i> SuSy3
SEQ ID NO: 5	EHGIVNNWDDMEKIW	<i>Drosophila melanogaster</i> Actin
SEQ ID NO: 6	EHGIITNWDDMEKIW	<i>Drosophila melanogaster</i> Actin
SEQ ID NO: 7	EHGIVKDWNDMERIW	<i>Drosophila melanogaster</i> ARP1
SEQ ID NO: 8	ENGIVRNWEDAHEVW	<i>Drosophila melanogaster</i> ARP2
SEQ ID NO: 9	GDRVLSRLHSVRERIGK	SS1 inactive Control peptide
SEQ ID NO: 10	GIVRKWISRFEVWPYLKK	SS2 active peptide SuSy 377-392
SEQ ID NO: 11	ILRVPFRTENGIVRK	SS11 inactive peptide
SEQ ID NO: 12	GIVRKWISRFEVWPYL	SS12 active synthetic peptide
SEQ ID NO: 13	GIVRKAISRFEVAPYL	SS15 less active synthetic peptide
SEQ ID NO: 14	SRFEVWPYL	SS16 less active synthetic peptide
SEQ ID NO: 15	GPTLKRTASTAFMNTTSKK	NR11 inactive synthetic peptide

SEQ ID NO: 16 GRMRRIATVEMMKK

SP26 inactive synthetic peptide

SEQ ID NO: 17 WISRFEVW

SMIN

[009] The active peptides listed above cause cells to adopt a denser F-actin meshwork to form, whereby the organization of filamentous actin is changed. Upon addition of the peptides to normal fibroblast cells, the peptides block the depolymerization of F-actin, causing F-actin to adopt an abnormal spiky morphology. The peptides are also useful in blocking cell division resulting in a decreased number of cells after *in vitro* peptide treatment.

[010] The peptides of the present invention can be used in cell culture or as a pharmaceutical drug to control diseases that involve uncontrolled cell division, such as cancer. The present peptides also provide a method for preventing migration of cells and can be used to prevent such migratory behavior as metastasis of cancer cells.

[011] The present peptides also provide a method for blocking cell motility, in particular those behaviors and movements involved in "rocket-based motility." "Rocket-based motility" is the term used to describe the activity of such highly pathogenic bacteria such as *Listeria* which rely on actin comet tails to provide the driving force for movement from cell to cell. The present peptides could be used as part of treatment to block motility and thus stop the spread of such bacteria.

[012] In general, the present peptides as recited above will provide stabilization of microfilaments as measured by inhibition of actin depolymerization at concentrations of at least 0.1mM up to 10mM of peptide. The present peptides may be formulated according to known pharmaceutical technology. They may be administered singly or in combination, and may further be administered in combination with other cancer or actin-binding drugs. They may be conventionally prepared with excipients and stabilizers in sterilized, lyophilized powdered form for injection, or prepared with stabilizers and peptidase inhibitors of oral and gastrointestinal metabolism for oral administration. Alternatively, the peptides can be prepared with additives or fused to carrier molecules that would increase peptide efficacy and cell entry. Peptides

containing naturally occurring amino acids may be produced intracellularly by introduction of DNA or RNA constructs.

### **BRIEF DESCRIPTION OF THE DRAWINGS**

[013] **Figure 1** is a listing of sequences discussed in the specification showing a sequence comparison of actin, sucrose synthase and the peptides of the invention.

[014] **Figure 2** is a series of microphotographs showing actin organization in human mammary epithelial tumor cells results in actin bundling to form long filapodia extending from the cell surface (Fig. 2B) rather than the lamellipodia characteristic of crawling tumor cells (Fig. 2A).

[015] **Figure 3** is a series of color photographs of uninjected *Xenopus* blastomeres (Fig. 3A), upon injection of inactive peptides (Fig. 3B), and active peptide (Fig. 3C), and microphotographs of the blastomere cleavage furrows with actin stained with rhodamine-phalloidin in uninjected normal embryos (Fig. 3D), and in embryos injected with the inactive peptide (Fig. 3E) and with the active peptide (Fig. 3F).

[016] **Figure 4** is a series of six microphotographs of *in vitro* polymerized actin comet tail formation. Rhodamine-labeled actin was added to cytoplasmic extracts shown in Fig. 4A. Addition of active peptides SS2 and SS12 caused complete disruption of the comet tails as shown in Fig. 4C, 4D and 4F.

[017] **Figure 5** is a series of electron micrographs of rabbit muscle actin bundled by co-polymerization with SS12 active peptide in a molar ratio of peptide:actin = 2 showing the nodulated shape of the filaments. Fig. 5A and 5C are controls; Figs. 5B and D show effects of addition of SS12.

[018] **Figures 6A-6F** are color photographs of electrophoretic gels of fractions after the addition of the peptides to *in vitro* actin to determine the actin bundling activity of the peptides.

[019] **Figure 7** is a color photograph of stained gels of G-actin and F-actin showing that the bundling activity of SS2 is not affected by the addition of phalloidin.

## **DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT**

### **Definitions**

[020] The term “peptide” herein refers to an amino acid sequence between 2 and 100 amino acids in length, the amino acids being joined by peptide linkages. The amino acids may be naturally and non-naturally occurring.

[021] The terms “derived from” or “based on” herein refers to, regarding a peptide amino acid sequence, having a relationship to a native sequence of a plant-specific enzyme.

[022] The term “substantially identical” is herein used to mean having an amino acid sequence which differs only by conservative amino acid substitutions or by non-conservative amino acid substitutions, deletions, or insertions located at positions which do not destroy the biological activity of the peptide.

[023] The term “homology” or “homologous” herein refers to an amino acid sequence similarity measured by the program, BLAST (Altschul et al (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402 and expressed as -(% identity n/n). In measuring homology between a peptide and a protein of greater size, homology is measured only in the corresponding region; that is, the protein is regarded as only having the same general length as the peptide, allowing for gaps and insertions using default values.

[024] The term “cell” herein refers to human, other vertebrate, insect, bacterial, plant, yeast, or other unicellular organisms.

[025] The term “motility” herein refers to cell behavior and movement. The term also refers to spontaneous and/or non-directional movement of cells – for examples, lamellipodial ruffling, crawling around the culture surface. Migration is a type of motility and cell migration and motility can be indicative of metastatic potential. The term “migration” herein refers to cell movement such as the crawling of cells from one tissue to another tissue, from tissue to blood

stream to tissue, or (in the lab setting) from one side of a filter to the other. The term also describes directional migration and movement.

[026] The term "metastatic potential" herein refers to the probability or potential spread of a disease from the organ or tissue of origin to another part of the body. The term also herein generally refers to the transmission of pathogenic microorganisms or cancerous cells from an original site to one or more sites elsewhere in the body, usually by way of the blood vessels or lymphatics.

[027] The term "effective amount" herein refers to an amount sufficient to elicit the desired biological response.

[028] The term "supra-additive" when used to refer to an effect from a combination of agents, herein refers to a total effect which is greater than the sum of the effects due to each of the individual agents.

[029] The term "subject" herein refers to any vertebrate species. Particularly preferred subjects are mammals, with humans being the most preferred subject.

[030] The term "conservative substitution" means a substitution where an amino acid residue is substituted for another in the same class, where the amino acids are divided into non-polar, acidic, basic and neutral classes, as follows: non-polar: Ala, Val, Leu, Ile, Phe, Trp, Pro, Met; acidic: Asp, Glu; basic: Lys, Arg, His; neutral: Gly, Ser, Thr, Cys, Asn, Gln, Tyr. A non-conservative amino acid substitution is one where the residues do not fall into the same class, for example, substitution of a basic amino acid for a neutral or non-polar amino acid.

### Introduction

[031] Sucrose Synthase, an enzyme in higher plants with important roles in carbohydrate partitioning was found to be associated with the actin cytoskeleton (Winter et al. *FEBS Lett.* 1998 Jul 3;430(3):205-8). In order to identify the actin binding site on Sucrose Synthase, a sequence of Sucrose Synthase with high homology to actin itself was identified. Synthetic peptides were derived from the consensus sequence, having similarity to the part of the

actin sequence. The active peptides of the invention change the organization of the actin cytoskeleton of animal actin *in vitro* and *in situ*, possibly by binding specifically to one of the actin:actin binding sites. Thus, these peptides are not only useful as a powerful tool to study regulation of actin polymerization and function in cells, but also may be useful as chemotherapeutic agents. The active peptides of the invention also offer the prospects of tumor cell specific targeting, where the peptide may be synthesized intracellularly following transfection by a vector encoding the peptide. Such a vector may be designed using nucleic acid sequence encoding, e.g., peptide SS2, using the codons described.

#### Sequences and Sequence Analysis of Sucrose Synthase

[032] Several actin binding proteins have been shown to display sequence and/or structural homology to segments of actin itself. See Tellam et al., *Trends Biochem Sci.* 1989 Apr; 14(4):130-3. In order to polymerize into double helical filamentous structures, each actin monomer must bind to four adjacent actin monomers, which led to the assumption that each actin monomer must have at least 4 different actin binding sites conformationally active upon binding and hydrolysis of ATP. It was therefore postulated that this pattern of sequence similarity between actin and actin binding proteins reflects the molecular mimicry by these actin filament capping/severing proteins of a site on actin involved in actin-actin interactions (Tellam et al. *Trends Biochem Sci.* 1989 Apr; 14(4):130-3; Puius et al., *Curr Opin Cell Biol.* 1998 Feb; 10(1):23-34). Classes of actin capping, severing or crosslinking proteins share homology with segments of the actin structure itself and in some cases these sequences have been shown to be involved in the association of those proteins with monomeric or filamentous actin (Tellam et al. 1989).

[033] The sequence homology of SuSy, actin related proteins (ARPs), actin and the specific activity of the synthetic peptides on the organization of actin filaments *in vitro* and *in situ* suggests a specific binding site of SuSy and ARPs shared with actin itself on the lateral site of actin filaments. In order to regulate the assembly of actin or the motility based on actin filaments or polymerization, association of the proteins to actin are regulated by affinity,

posttranslational modification or effectors. While interaction with other proteins (i.e. ActA) might be a regulatory factor between capping, nucleating and branching activity of the Arp2/3 protein, binding of SuSy to actin is metabolically induced by high concentrations of its substrate sucrose or maltose.

[034] In order to identify the actin binding site on SuSy, a sequence similarity analysis with BLAST (Altschul et al. 1997) was carried out and the SuSy sequence was compared with known actin binding sites from other proteins (Tellam et al. 1989). A database search revealed a high homology between the following sequences: sequences of SUS1 and SUS2, SS2 (SEQ ID NO: 10), actin itself and actin-related proteins as described in Example 1 and shown in Fig. 1.

[035] Table 1 shows the sequence alignment between 15 residue homologous sequences of three isoforms of SuSy isolated from *Zea mays* and with actin and actin-related proteins (ARP) from *Drosophila melanogaster* (Dro me). Conserved amino acid substitutions are marked with an asterisk (\*) in the consensus sequence. The absolutely conserved residues are indicated and identified, using the numbering of SEQ ID NO: 1, as E1, G3, I4, W8 and W15.

**Table 1**

SEQ ID NO	Protein	Species	Position	Homologous sequence
SEQ ID NO: 2	SuSy 1	Zea mays	367-381	ENGILRKWISRFDVW
SEQ ID NO: 3	SuSy 2	Zea mays	375-389	ENGIVRKWISRFEVW
SEQ ID NO: 4	SuSy 3	Zea mays		ENGILKKWISRFDVW
SEQ ID NO: 5	Actin	Dro me	73 - 87	EHGIVNNWDDMEKIW
SEQ ID NO: 6	Actin	Dro me	73 - 87	EHGIITNWDDMEKIW
SEQ ID NO: 7	ARP1	Dro me	76 - 90	EHGIVKDWNDMERIW
SEQ ID NO: 8	ARP2	Dro me		ENGIVRNWEDAEHVW
SEQ ID NO: 1			CONSENSUS	<b>E*GI**-W-----*W</b>

### Actin-Blocking Peptides

[036] Referring now to Fig.1 and Table 1, Sucrose synthases, actins and actin-related proteins were aligned to find the consensus sequence of SEQ ID NO: 1. Using the consensus sequence, different peptides were created.

[037] Short synthetic peptides, as used in this study, usually display random conformation in aqueous solutions. The specific effects of the synthetic peptides on actin polymerization *in vitro* and *in situ* indicate that the sequence of amino acids in this peptide either determine a specific secondary structure or are active independent of the conformation. Formation of actin bundles can be caused *in vitro* by a number of polycations and basic polypeptides, largely independent of the specific structure of the bundling agent used (Tang and Janmey, *J Biol Chem.* 1996 Apr 12; 271(15):8556-63).

[038] This consensus sequence has not been identified by analysis of crystal structures of actin, site-directed mutagenesis, mutant analysis in yeast or high-resolution electron micrographs, as being involved in the association of actin monomers to build filaments or bundles. The motif is not found in any other known actin binding proteins in searches using available programs such as ProSite and BLAST. Therefore this site seems to be unique to actin, ARPs and SuSy.

[039] The underlined portion of sequence in SS2, SS11, SS12, SS15 and SS16 and the sequence in green indicating a possible region of specificity, both appear to be necessary for acting-bundling activity.

[040] The specificity of the consensus sequence was further narrowed down by varying the synthetic peptides at the N-terminal (SS11) and C-terminal (SS16) portion of the peptide and then co-polymerizing the peptides with actin *in vitro* (see Table 2 and Fig. 6A-F for bundling activities). While the synthetic peptide of the N-terminal sequence (SS11) did not cause actin to bundle during *in vitro* polymerization, the C-terminal segment alone (SS16) bundled actin, but with reduced affinity. A similar effect was observed with a synthetic peptide where the two conserved tryptophans were substituted by alanine (SS15). The alignment in Table 2 further

demonstrates that certain residues such as the tryptophans, W8 and W15, may be necessary for active peptides, as shown at SEQ ID NO: 17, Table 2. The effective peptide to actin ratio for bundling for SS16 was >16:1 and for SS15, about 16:1, as opposed to 1:1 for SS2 and SS12, the most preferred embodiments.

[041] Clusters of basic amino acids in synthetic peptides have been shown to affect actin polymerization (Tang and Janmey, *J Biol Chem.* 1996 Apr 12; 271(15):8556-63). The possibility of a charge-related effect with a synthetic peptide containing two "double-basic clusters" (SP26) was tested. This peptide was not able to bundle actin filaments *in vitro*. Peptides, such as SS11 and SP26 (Fig. 1), containing "basic clusters" similar to the motif were not effective in bundling, while the addition of basic residues, such as in SS2 (Fig. 1), did not increase the bundling activity.

[042] Combining the above information, it appears that all amino acids which are identical or conserved between SuSy, ARPs and Actin sequences (Fig.1) are essential for bundling at high affinity, i.e. the peptides should be highly homologous to the consensus sequence SEQ ID NO: 1 and the sequence E-x-G-[IV]-[IVL]-x(2)-W-x(5)-[IV]-W (SEQ ID NO: 18).

[043] The consensus sequence derived from sequences of homology of sucrose synthase, actin and actin-related proteins can be used to direct the synthesis of peptides that possess actin-binding properties similar to SuSy and actin-related proteins. Table 2 shows synthetic peptides that were made based on the consensus sequence, SEQ ID NO: 1, of Table 1. Table 2 shows the different peptides and aligns them relative to the Control SS1 peptide. The preferred embodiments are the active peptides, SEQ ID NOS: 10 and 12.

Table 2

SEQ ID NO.	synthetic peptide	Sequence	<i>In vitro</i> actin bundling activity
SEQ ID NO: 9	SS1	GDRVSRLHSVRERIGK	CONTROL
SEQ ID NO:10	SS2	GIVRKWISRFEVWPYLKK	active
SEQ ID NO:11	SS11	ILRVPPFRTENGIVRK	inactive
SEQ ID NO:12	SS12	GIVRKWISRFEVWPYL	active
SEQ ID NO:13	SS15	GIVRKALSRFEVAPYL	less active
SEQ ID NO:14	SS16	SRFEVWPYL	less active
SEQ ID NO:15	NR11	GPTLKRTASTAFMNTTSKK	inactive
SEQ ID NO:16	SP26	GRMRRIATVEMMKK	inactive
SEQ ID NO:17	SMIN	WISRFEVW	less active

[044] The peptides may be made and purified by methods known in the art, preferably by *in vitro* automated synthesis, but also by recombinant DNA methods. Furthermore, these peptides can be synthesized using L-amino acids and selected non-natural or other modified amino acids, as is known in the art, in order to synthesize peptides which can act upon targets in the body and be degraded, yet do not interfere with normal protein function. The peptides can be stored in lyophilized form and dissolved in aqueous buffers or water prior to use. For the purposes of experimental use, the peptides are dissolved in sterilized degassed buffers to optimize biological activity to remain stable over 1-3 months at 4° C.

[045] Suitable buffers or diluents should be capable of solubilizing the active peptide, preferably below pH 8 to prevent the peptide from precipitating out of solution too easily.

[046] The present peptides are based on proteins and enzymes having actin binding capability and are substantially homologous to SEQ ID NOS: 10 and 12 (SS2 and SS12 active peptides). Accordingly, they may be from about 13 to 100 amino acids in length, preferably 15-20 amino acids in length, more preferably 13-15 amino acids in length. The peptide

subsequences can be extended in either the amino and carboxy direction or both, with the sequence from the native protein from which the peptide was derived.

[047] When extending the peptides, in one embodiment, beyond the active synthetic peptide in the amino and/or carboxy directions, it is preferred that the sequence of the native *Zea mays* Sucrose Synthase 1, as set forth in GenBank Accession Number: P04712, native *Zea mays* Sucrose Synthase 2 and its isotypes, as set forth in GenBank Accession Number: P49036 and 2008300A, or the native sequence of the novel *Zea mays* Sucrose Synthase 3 at GenBank Accession Number: gi:22121990, be used. In a separate preferred embodiment, the sequences of a native ARP2 protein from *Drosophila melanogaster* is used to extend the peptide. For example, GenBank Accession Number: P45888 (Fyrberg,C., Ryan,L., Kenton,M. and Fyrberg,E., Genes encoding actin-related proteins of *Drosophila melanogaster*, *J. Mol. Biol.* 241 (3), 498-503 (1994)).

[048] The extended sequence need not be identical to the recited sequences above, however it should be substantially identical, preferably at least 80% homologous, more preferably at least 90% homologous.

[049] It is further contemplated that the peptides are fused to a protein, signal sequence, peptide domain or other carrier molecule which would permit the entry of the SuSy peptides into mammalian cells. For example, the peptides can be made with the addition of Pep-1, a short amphipathic peptide carrier described in Morris, MC et al., *Nature Biotechnology* 19:1173-1176 (Dec 2001). Other methods of enhancing peptide delivery include but are not limited to, the linkage of competent signal peptides to the peptides, such as the NFκB sequence, described by Lin et al in U.S. Pat. No. 6,043,339 or the homeobox peptide, described by Fischer et al, in U.S. Pat. No. 6,472,507, covalently coupling the peptides to a nucleic acid-binding group, cationic lipids, dendrimers or other carrier molecules, or encapsulation of the peptides of the invention in liposomes, microparticles, or nanoparticles.

[050] The invention further contemplates the use of the peptides tagged with detectable agents including, but not limited to, antibodies, radioanalogs, products or compounds having

distinctive absorption, fluorescence, or chemi-luminescence properties, such as rhodamine, fluorescein, green fluorescent protein (GFP) or semiconductor nanocrystal beads. Peptides tagged with such detectable agents would be useful for studying and monitoring the peptides and their effect on actin and microfilaments.

#### Application of the Active Peptides

[051] The delivery of the preferred peptides of the invention into cells results in the stabilization of microfilaments and actin bundling.

[052] The active peptides SS2 and SS12 of the preferred embodiment were shown to cause actin bundling, thereby preventing or decreasing cellular activities involving actin including cytokinesis, cell division, the ability to form lamellipodia, ruffling, motility and movement, cytoskeleton support and cell structure.

[053] In one embodiment, the preferred peptides may result in the bundling of actin fibers by parallel binding. Referring to Fig. 2, upon the addition of the active peptides to normal fibroblast cells, the depolymerization of F-actin appears to have been blocked, causing F-actin to adopt an abnormal spiky morphology. Fig. 2 is a series of microphotographs showing actin organization in human mammary epithelial tumor cells results in actin bundling to form long fillapodia extending from the cell surface (Fig. 2B) rather than the lamellipodia characteristic of crawling tumor cells (Fig. 2A).

[054] In another embodiment, the peptides of the invention may also prove to be useful in blocking cell division as shown by Fig. 3. The injection of the active peptides of the invention to dividing cells will result in the inability to complete cell division through the stabilization of microfilaments. The peptides of the invention have a similar effect on actin as the compound, paclitaxel, which is widely used as an anti-tumor agent. Therefore, the peptides of the invention will likely find use as anti-tumor agent.

[055] Referring to Fig. 4, in another embodiment, the present peptides also provide a method for blocking cell motility, in particular those behaviors and movements involved in "rocket-based motility." "Rocket-based motility" is the term used to describe the activity of such

highly pathogenic bacteria such as *Listeria* which rely on actin comet tails to provide the driving force for movement from cell to cell. The present peptides could be used as part of treatment to block motility and thus stop the spread of such bacteria.

[056] In another embodiment, the active peptides of the invention cause cells to form a denser F-actin meshwork, with the F-actin decorated as shown in Fig. 5. The addition of the active peptides to cells results in changing the organization of filamentous actin and increasing the amount of at least some proteins bound to actin *in vitro*.

[057] The present active peptides as recited above will provide stabilization of microfilaments and inhibition of *in vitro* actin depolymerization at a concentration of preferably at least 1:1 molar ratio of the peptide to actin, as demonstrated in the Examples and shown in Fig. 6A-6F. In a preferred embodiment, the peptides will result in approximately 100% stabilization of microfilaments *in vitro* at a 1:1 molar ratio of peptide to actin.

[058] It will be apparent to those of skill in the art that the therapeutically effective amount of DNA or polypeptide of this invention will depend, *inter alia*, upon the administration schedule, the unit dose administered, whether the peptide or DNA is administered in combination with other therapeutic agents, the immune status and health of the patient, and the therapeutic activity of the peptide or DNA. The present peptides may be prepared according to known pharmaceutical methods. They may be administered singly or in combination, and may further be administered in combination with other chemotherapeutic drugs. The peptide can be conventionally prepared with a pharmaceutically acceptable additive, carrier, diluent, solvent, filler, lubricant, excipient, binder or stabilizer.

[059] In a preferred embodiment, the peptides are administered by intratumoral injection at the situs of the cancer. One skilled in the art would understand and be able to use such methods of intratumoral injection of anti-tumor agents as disclosed by Flashner-Barak in U.S. Pat. No. 6,569,459 and hereby incorporated by reference. It is further contemplated that the peptides can be administered intravenously, systemically or orally in the form of a tablet, lozenge, capsule, powder, aqueous or oily suspension, syrup, elixir, implant or aqueous solution.

In accordance with good clinical practice, it is preferred to administer the composition at a dose that will produce anticancer effects without causing undue harmful side effects.

[060] The invention encompasses the delivery of the active peptides to tumors or regions of interest using various suitable delivery methods including, but not limited to, biodegradable microcapsules or immuno-stimulating complexes (ISCOMs), cochleates, polymers, microgels, slow-release molecules, or liposomes. The invention further contemplates the delivery of a polynucleotide encoding said active peptides having sequences obtained, e.g. by inputting peptide sequences into a reverse translation program such as "molecular tool kit" maintained by R. Bowen at Colorado State University. Such nucleic acids can be prepared using various suitable gene preparation and delivery methods including, but not limited to, genetically engineered attenuated live vectors such as viruses or bacteria, recombinant (chimeric) virus-like particles, e.g., bluetongue, or constructs and vectors containing the polynucleotide, and so-called "naked DNA." The amount of adjuvant employed will depend on the type of adjuvant used. For example, when the mucosal adjuvant is cholera toxin, it is suitably used in an amount of 5 mg to 50 mg, for example, 10 mg to 35 mg. When used in the form of microcapsules, the amount used will depend on the amount employed in the matrix of the microcapsule to achieve the desired dosage. The determination of this amount is within the skill of a person of ordinary skill in the art.

[061] The peptides of the invention are administered in amounts readily determined by persons of ordinary skill in the art. The following is meant to act as guidance. For adults a suitable dosage will likely be in the range of 10 mg to 10 g, preferably 10 mg to 100 mg. A suitable dosage for adults will also be in the range of 5 mg to 500 mg. Similar dosage ranges will be applicable for children. Those skilled in the art will recognize that the optimal dose may be more or less depending upon the patient's body weight, disease, the route of administration, and other factors. Those skilled in the art will also recognize that appropriate dosage levels can be obtained based on results with known oral vaccines such as, for example, a vaccine based on an *E. coli* lysate (6 mg dose daily up to total of 540 mg) and with an enterotoxigenic *E. coli* purified

antigen (4 doses of 1 mg) (Schulman et al., *J. Urol.* 150:917-921 (1993); Boedecker et al., *American Gastroenterological Assoc.* 999: A-222 (1993)). The number of doses will depend upon the disease, the formulation, and efficacy data from clinical trials. Without intending any limitation as to the course of treatment, it is contemplated that the treatment using the peptides can be administered over 3 to 8 doses for a primary immunization schedule over 1 month (Boedecker, *American Gastroenterological Assoc.* 888: A-222 (1993)).

[062] While it is known that amino acid peptides tend to degrade quickly, the effects of the active peptides of the invention were found by the inventors to have effects on cell division as long as 36 hours upon administration of the peptide. In electroporation experiments, using cells having a 24 hr cell cycle division, within 36 hours there were decreased number of cells.

[063] The following examples are provided as exemplary of the invention and should in no way be seen as limiting the invention to these specific examples.

#### EXAMPLE 1

##### Sequence Analysis and Structural Comparison of Sucrose Synthase

[064] Referring now to Fig. 1, use of BLASTP 2.0.8 revealed high homology between Sucrose Synthase sequence (SS2) with actin itself and actin-associating proteins. Fig. 1 shows that *Zea mays* Sucrose Synthase 2 (SUS2) residues 375-389 (SEQ ID NO: 3) have significant homology with *Zea mays* Sucrose Synthase 1 (SUS1) at residues 367-381 (SEQ ID NO: 2), with an expectancy score of 4e-04, 86% identical and 99% positively aligned. SUS2 has an expectancy score of 19, with 40% identity and 60% positives when aligned with residues 54-68 of *Zea mays* Actin (GenBank Accession No.: 1498382 (U60507)). Figure 1 further shows a sequence similarity between the SUS1 and SUS2 sequences and a consensus sequence of various actin proteins. This indicates the presently recognized possibility of a binding site on actin for a peptide having sequence similarities in this region. Shown below the Actin consensus sequence are various synthetic peptides made according to the teachings of the present specification and the activity of those peptides. As discussed below, the most active peptides, SS2 and SS12

contained the underlined subsequence GIVRWK, which appears to be necessary, but not sufficient, for activity.

## EXAMPLE 2

### Making Active Peptides

[065] Using the consensus sequence in Table 1 (SEQ ID NO: 1) which was derived from the aligned sequences of SuSy1, SuSy2, SuSy3 and actin, active synthetic peptides that bind and alter F-Actin polymerization were created. The dashed lines represent any amino acid, and the symbol (\*) represents amino acids that are conservative substitutions. Table 2 shows different variations of the peptides and their corresponding level of actin bundling. The peptides are soluble at lower pHs and preferably stored in low salt buffer (LSB: 5 mM Tris-HCl, pH 8.0, 0.2 mM CaCl<sub>2</sub>, 0.2 mM ATP, 0.5 mM DTT).

[066] The peptides were synthesized through commercial oligosynthesis. Peptides were then lyophilized and resuspended in pH 8.0 buffer Tris-HCl at 1mM concentration.

## EXAMPLE 3

### Active Peptides Stabilize Microfilaments and Inhibit Cell Division

[067] In this example, the active peptides were used to control cell division. The cells treated with the active peptide SS12 showed a decrease in the number of cells after 24 hours compared to the cells treated with the inactive peptide. The cell division of the SS12 treated cells was inhibited for the 36 hours the cells were under observation.

[068] Electroporated cells were treated with fluorescent dextran plus peptide so those cells receiving the peptide could be identified. Typically a very small number of cells successfully incorporate substance during electroporation, making it difficult to identify those receiving peptide. Typically one sees 10-20% incorporation. But, there is good evidence showing that if a cell incorporates one component in buffer, it will incorporate all components. Thus, dextran can be used to identify those cells receiving peptide. After electroporation of cells with fluorescent dextran and the active peptide SEQ ID NO: 12, the dextran-labeled cells were initially counted and then incubated for 18 hrs. After incubation overnight, the dextran-labeled

cells were once again counted. The following Tables 3 and 4 show the average change in dextran-labeled cells after the cells are electroporated and the peptide has invaded the cell body. The cell numbers of the SS12 treated peptide seemed to fluctuate after incubation with various concentrations of the SS12 peptide. While there may be no actual decrease in the number of cells, in general it appears that the SS12 peptide treated cells did not exhibit the expected increase in cell population as observed with the SS1 control peptide treated cells.

Table 3. SS12 active peptide-electroporated cells, 6 dishes:

Concentration peptide	% change in cell number
0.2 $\mu$ M	+ 13.21
1.0 $\mu$ M	- 1.5
10.0 $\mu$ M	- 8.6
1.0 mM	+ 2.4
1.0 mM	- 6.9
1.0 M	- 12.5

Table 4. SS1 control-electroporated cells, 6 dishes:

Concentration peptide	% change in cell number
0.2 $\mu$ M	+ 1.2
1.0 $\mu$ M	+ 17.4
10.0 $\mu$ M	+ 43.8
1.0 mM	+ 64.9
1.0 mM	+ 38.4
1.0 M	+ 45.0

[069] In further experiments, cytokinesis but not nuclear division was blocked in tumorigenic cells by addition of the active peptide SS12. MCF7 cells were electroporated with GFP-actin and imaged (image not shown). After addition of peptide in DMSO, the MCF7 cells were continuously imaged for up to 4 hours. The cells that incorporated the active peptide exhibited two nuclei but could not divide by cytokinesis. The actin filaments within the cells were observed by fluorescence and had the appearance of spikes. Thus it appears that treatment by the active peptide results in inhibition of cytokinesis and blocks cell division.

#### **EXAMPLE 4**

##### **Actin Reorganization After Peptide Treatment**

[070] Referring now to Fig. 2, it can be shown that treatment with an active peptide results in actin organization in human mammary epithelial tumor cells. MDA 231 cells were electroporated with SS1 (panel A) or SS12 (panel B), fixed, and rhodamine-phalloidin stained. Control epithelial tumor cells were treated with SS1 (SEQ ID NO: 9) resulting in no effect on actin-filled lamellipodia (or ruffling) characteristic of crawling cells. These normal cells treated with the inactive peptide are shown in Fig. 2A after treatment.

[071] The MDA 231 cells treated with the peptide SS12 (SEQ ID NO: 12) are shown in Fig. 2B. MDA 231 cells are a particularly aggressive tumor cell line crawling and moving from between tissues. After treatment with the peptide, the cells no longer have lamellipodia; however they do exhibit numerous long spiky structures and filopodial-like structures extending from the cell surface. Eventually, these cells ceased movement due to the bound F-actin.

#### **EXAMPLE 5**

##### **Effect of Peptides on Actin-Dependent Cleavage During Early Development**

[072] Referring now to color photographs of Fig. 3 the peptides have a severely abnormal effect on the embryonic development of cells. Embryos from *Xenopus laevis* were injected with the inactive control peptide of SEQ ID NO: 9 and the active peptide of SEQ ID NO: 10. Fig. 3A shows normal, uninjected embryos at the 8-cell stage (only 4 blastomeres can be seen; the other 4 cells are directly beneath them.) The same concentration of the inactive peptide was injected into embryos and had no effect as shown in Fig. 3B. The embryos resemble the uninjected normal embryos of Fig. 3A.

[073] Fig. 3C shows embryos that had been injected with 0.6 mg/mL (final concentration in egg) of the active peptide, SS2, and fixed at the same time period. When injected into the embryos, the SS2 peptide appeared to block several cell divisions, which typically are an hour apart. This demonstrates that the SS2 peptide does not degrade quickly but

has a lasting effect on cell division for at least 3 divisions. The resulting blastomeres were unable to regain function and were severely abnormal showing abortive cleavage furrows.

[074] The three bottom panels of Fig. 3 shows the effect of the active peptide on actin organization in embryos from *Xenopus laevis* as detected with rhodamine phalloidon, which labels filamentous actin. The uninjected normal embryos (Fig. 3D) and the embryos injected with the inactive peptide (Fig. 3E) successfully completed three divisions, forming 8-cell embryos with no residual filamentous actin seen between the blastomeres. However, in the embryos injected with the active peptide (Fig. 3F), filamentous actin is seen in the abortive cleavage furrows. Cleavage furrows had been initiated, suggesting actin bundling had occurred, but cytokinesis was blocked.

#### EXAMPLE 6

##### Effect of Peptides on Actin Comet-tail Formation in Cytoplasmic Extracts

[075] Referring now to Fig. 4, this example demonstrates that the present active peptides affect actin comet-tail formation in cytoplasmic extracts. In Fig. 4A and 4B, *in vitro* polymerized actin forms comet tail from rhodamine-labeled actin added to *Xenopus laevis* egg cytoplasmic extract. This was set up according to the protocol in Taunton et al., *J Cell Biol.* 2000 Feb 7; 148(3):519-30. The addition of the active peptide of SEQ ID NO: 3 (SuSy2) caused complete disruption of the comet tails as shown in Fig. 4C and 4D.

[076] As described by Taunton et al. in *J Cell Biol.* 2000 Feb 7; 148(3):519-30, *Xenopus* Protein Kinase C (Chen et al. *Second Messengers Phosphoproteins.* (1998) 12:251-26), was cloned upstream of enhanced green fluorescent protein (GFP) (Heim et al., *Nature* 373:663-664, 1995), in the *Xenopus* expression vector CS2+. The resulting fusion protein, XPKC-GFP, is enzymatically active *in vitro* and in cultured cells is recruited to the plasma membrane in response to PMA, which is phorbol 12-myristate 13-acetate, a potent diacylglycerol mimetic that acts as an unspecific protein kinase activator (Sheldahl et al., *Curr. Biol.* 9:695-698 1999). PMA also plays a major role in the major cortical events of fertilization, including granule exocytosis, resumption of membrane trafficking, contraction of the cortex, and cleavage furrow

formation (Bement and Capco, *J. Cell Biol.* 108:885-892 1989; Bement and Capco, *Proc. Natl. Acad. Sci. USA.* 88:5172-5176, 1991).

[077] Approximately 5 nl of XPKC-GFP RNA and 20 nl of a stock solution of rhodamine-labeled non-muscle actin (10 mg/ml in 2 mM Tris-HCl [pH 8.0], 0.2 mM CaCl<sub>2</sub>, 0.2 mM ATP, and 0.5 mM DTT; Cytoskeleton) were injected into manually defolliculated stage VI oocytes. After 8–10 h, meiotic maturation was triggered by the addition of 1 µg/ml progesterone, and oocytes were incubated overnight in Barth's medium at 16–17°C. Several hours after germinal vesicle breakdown, oocytes were activated by pricking with a glass micropipet. Oocytes were mounted in viewing dishes for live cell analyses as described previously (Rowning et al., *Proc. Natl. Acad. Sci. USA.* 94:1224-1229, 1997; Larabell, *Methods Mol Biol.* 2000;135:175-82, 1998), using a BioRad MRC 1024 confocal laser scanning microscope equipped with a Nikon Diaphot 200 microscope and a Nikon 60x PlanApo 1.4 NA oil immersion lens. XPKC-GFP constructs and rhodamine-actin were visualized using fluorescein and rhodamine filters, respectively. Optical sections from the outer 20 µm were collected. For a given time-lapse sequence, multiple images of a single optical section were collected as rapidly as possible. Images were collected using a 512 x 512-pixel format at 1 frame per 3.5 sec for up to 1 hour. Data were analyzed using IMAGESPACE software (Molecular Dynamics, Piscataway, NJ) on a Silicon Graphics computer.

#### Whole Mount Immunocytochemistry

[078] Eggs were collected from adult frogs and fertilized as described previously by Rowning et al., *Proc. Natl. Acad. Sci. USA.* 94:1224-1229, 1997. Briefly, adult frogs (*Xenopus laevis*) were raised in the laboratory and fed trout chow (Purina) twice a week. Ovulation was induced by injecting 800 units of human chorionic gonadotropin (Sigma Chemicals, St. Louis, MO) into the dorsal lymph sac of each frog. Approximately 12 h later, eggs were stripped into a dry Petri dish and fertilized by overlaying them with a suspension of approximately one-eighth of a minced testis in 1-2 ml of one-third strength modified amphibian Ringer's solution (1/3 MR) (100% MR = 100 mM NaCl, 2 mM KCl, 1 mM MgCl<sub>2</sub>, 2 mM CaCl<sub>2</sub>, 50 µg/ml gentamycin, and

5 mM Hepes adjusted to pH 7.2 with NaOH). Fertilization was allowed to proceed for 6-8 min before de-jellying eggs with 2.5% cysteine hydrochloride in 1/3 MR adjusted to pH 8.0 with NaOH. Eggs were then rinsed 3-5 times with fresh 1/3 MR. They were fixed overnight in 4% paraformaldehyde, 0.1% glutaraldehyde, 100 mM KCl, 3 mM MgCl<sub>2</sub>, 10 mM HEPES, 150 mM sucrose, and 0.1% TRITON X-100 (pH 7.6). Nonspecific binding was blocked by incubation (1-2 h, with rotation) in 0.1% TRITON X-100 in Super Block (Pierce Biotechnology, Rockford, IL). Specimens were then incubated for 1 h with 0.16  $\mu$ M fluorescein-phalloidin in Super Block. Eggs were viewed with a BioRad 1024 confocal laser scanning microscope using a fluorescein filter.

#### Cell-free Reconstitution of Vesicle Movement

[079] Crude *Xenopus* egg extract was prepared as described except that cytochalasin D was omitted (Murray and Kirschner, *Nature*. 1989 May 25; 339(6222):275-80). Cytosol and a heavy membrane fraction were prepared by centrifugation of the crude extract (2 h, 300,000 g max; BECKMAN SW-50 rotor). The viscous glycogen pellet beneath the membrane layer was discarded. Cytosol was further clarified by spinning for 15 min at 541,000 g max (BECKMAN TLA 100.3 rotor). Crude membranes (diluted with one volume of 2 M sucrose) and cytosol were snap frozen in separate aliquots for storage at -80°C unless otherwise indicated. Clarified cytosol contained a small population of vesicles, but motility assays were far more robust (more comet tails over a longer time period) when cytosol was supplemented with the heavy membrane fraction.

[080] For the standard assay, crude membranes in 1 M sucrose (20- $\mu$ l aliquot, derived from ~80  $\mu$ l crude extract) were washed and resuspended in 14  $\mu$ l buffer A (50 mM NaCl, 5 mM MgCl<sub>2</sub>, and 50 mM tris [pH 7.5]). To the membrane suspension were added 35  $\mu$ l cytosol (derived from ~80  $\mu$ l crude extract), 0.3  $\mu$ l rhodamine-labeled actin (prepared according to Kellogg et al., *Development*. 1988 Aug; 103(4):675-86), 3  $\mu$ l PMA (20  $\mu$ M stock in 10% DMSO), and 20  $\mu$ l buffer A at 4°C. 20- $\mu$ l samples were then warmed to room temperature. After 30-40 min, 2- $\mu$ l aliquots were viewed with a Nikon E800 microscope to yield the

photomicrographs of Fig. 4. Images were acquired with a Princeton Instruments cooled CCD camera (standard rhodamine filter set, Princeton Instruments, Trenton, NJ) and analyzed with WINVIEW (Visitron System, Puchheim, Germany) or METAMORPH software (Princeton Instruments, Trenton, NJ). Pixels having an intensity over a threshold value (set to a value greater than the background fluorescence in regions devoid of comet tails) were summed over 10 random fields to quantitate relative actin assembly.

[081] Time-lapse sequences showing actin comet tails in *Xenopus* egg extracts are shown in Fig. 4. Cytosol was supplemented with a crude membrane fraction, rhodamine-actin, 1  $\mu$ M PMA and peptide SS2 or SS12 (1 $\mu$ M). After incubating at room temperature for 20 min, samples were viewed by fluorescence. Actin comet tails formed in *Xenopus* cytoplasmic extracts where no SS2 or SS12 peptides were added as shown by the photographs A,B and E of Fig. 4. In extracts where the SS2 or SS12 peptides were added, Fig. 4 C, D and F, little or no actin comet tail formation was observed.

[082] This example demonstrates that the active peptide can be used for blocking cell motility, in particular those behaviors and movements involved in "rocket-based motility." Highly pathogenic bacteria such as *Listeria* rely on actin comet tails to provide the driving force for movement from cell to cell. The SS12 peptide could be used as part of treatment to stop the spread of such bacteria.

#### EXAMPLE 7

##### Actin Bundling and Decoration after Polymerization in Presence of SS12 Peptide

[083] The actin bundles caused by co-polymerization of SS12 peptide with purified rabbit muscle actin *in vitro* were visualized by negative stain electron microscopy (Fig. 5). The bundles still show overall filamentous structure about 3 times the width of actin filaments polymerized *in vitro* in the absence of SS12 peptide (Fig. 5A, B). The surface of the bundles is not smooth, but irregular, resembling attempts to branch or attachments of short actin bundles to the sides of the long ones (Fig. 5C). The peptide-bundled actin filaments are much shorter than

the control filaments as visualized with rhodamine-phalloidin by fluorescence microscopy (data not shown).

[084] Purified rabbit actin was polymerized in the presence or absence of the SS12 peptide and by absorption applied to a carbon coated copper grid. Negative contrastation was carried out with Pb-citrate and the dried grid was viewed in an Electron microscope at high magnifications (12,000x and 65,000x). As shown in Fig. 5, polymerization in the presence of the peptide SS12 showed an increased diameter of the actin microfilaments compared to the controls. Higher magnification identified a "nodulated" decoration of the otherwise smooth filaments. The image of SS12-modified actin bundles suggests that the peptide mimicks an initiation of branching of the filaments.

[085] Upon binding to ActA, the Arp2/3 complex has enhanced nucleating activity for actin polymerization (Welch et al.). We tested a possible involvement of the homologous sequence on nucleating or capping activity by following the time-dependent fluorescence increase upon actin /pyrene-actin polymerization in the presence of the synthetic peptide SS12. SS12 did not show any nucleating or capping activity, but an increased fluorescence typical for an increased weight concentration of the pyrene-actin i.e. due to bundling of filaments (data not shown)

## EXAMPLE 8

### *In vitro* bundling of actin by Active Peptides

[086] The peptides in Table 2 were added to polymerised actin *in vitro* at different molar ratios. Centrifugation was used to separate fractions containing F-actin, G-actin and bundled actin, which were then run out on agarose gels.

[087] Purified rabbit muscle actin (Cytoskeleton Inc., Denver, MO) was depolymerized in Low Salt Buffer [LSB; 5 mM Tris-HCl, pH 8.0, 0.2 mM CaCl<sub>2</sub>, 0.2 mM ATP, 0.5 mM DTT, mM sucrose) for 1 h on ice and denucleated by centrifugation for 1 h at 100,000xg, 4°C. 13 µM monomeric actin was polymerized in vitro by addition of 1/50 vol. Polymerization Inducer (PI; 20 mM MOPS, pH 7.5, 2 M KCl, 50 mM ATP and 100 mM MgCl<sub>2</sub>) in the presence or absence

of molar ratios of the peptides to actin of 0 (no peptide); 0.5 (6.5  $\mu$ M peptide); 1 (13  $\mu$ M peptide); 2 (26  $\mu$ M peptide); 4 (52  $\mu$ M peptide); and 8 (104  $\mu$ M peptide) and incubated for 30 min at 30°C. The samples were centrifuged for 15 min at 10,000xg at room temperature to pellet “bundled filaments”. The resulting supernatant was centrifuged for 1 h at 100,000 xg at room temperature to separate F-actin filaments (G-actin) from “soluble” monomeric F-actin. The fractions were boiled in SDS-sample buffer and analyzed on a 10% SDS-Polyacrylamide gel stained with Coomassie.

[088] Referring now to Fig. 6A, the gel shows that the SS2 active peptide causes bundling of actin. SS2 active peptide was added to unpolymerized actin *in vitro* at a molar ratio of peptide to actin of 0, 0.5, 1, 2, 4, and 8. As shown, the active peptide SS2 causes bundling of F-actin and G-actin at a 0.5 molar ratios. Notice that at a molar ratio of 0.5, F-actin and bundled actin produce a similar size band, showing that at this molar ratio, the active peptide produces half-bundled and half-F-actin. Thus, it can be concluded that the *in vitro* dosage of peptide to bundle all the F-actin during polymerization, the SS2 peptide must be delivered at a 1:1 molar ratio of peptide:actin.

[089] Referring now to Fig. 6B, the gel shows the effect of SS2 on polymerized actin *in vitro* with molar ratio of 0, 0.5, 1, 2 and 10. Purified rabbit muscle actin (2 nmol) was polymerized with SS2 *in vitro* for 30 min (G-actin, lanes 2 and 3) and subsequently incubated with different concentrations of SS2-peptide (0, 1, 2, 4 and 20 nmol) for another 30 min to make F-actin (lanes 4-8). Only the highest concentration of SS2 caused bundling of filamentous actin (lane 8). When SS2 was present during polymerization (G-actin, lanes 2, 3), it caused bundled actin at a molar ratio peptide: actin of 2.

[090] Referring now to Fig. 6C, the gel shows bundling activity of SS2, SS11, S12, SP3 peptides. “PI” means “polymerization induced” by the addition of a higher concentration of ATP and potassium in buffer, and whether polymerization was induced is indicated by a “+” or if polymerization was not induced is indicated by a “—”. Even upon inducing polymerization, the SS11 and SP3 peptides do not produce bundled actin. The addition of the SS2 and SS12

peptides alone in a 1:1 molar ratio to actin each result in half of the actin bundled and half of the actin found in the F-actin fraction as fine and soluble actin filaments; however, upon inducing polymerization, the bulk of the actin is bundled and not found in the F-actin fraction.

[091] Referring now to Fig. 6D, the gel shows the bundling activity of SS15, SS2, SS16, SS12c peptides. Purified rabbit muscle actin (2 nmol) was polymerized *in vitro* for 30 min and subsequently incubated at molar ratio peptide:actin = 1:1. Even when polymerization was induced, the SS15 and SS16 peptides did not cause bundled actin, but the bulk of the actin is found in the G-actin or the F-actin fractions. The addition of the SS2 and SS12 peptides alone in a 1:1 molar ratio to actin each result in half of the actin bundled and half of the actin found in the F-actin fraction; however, upon inducing polymerization, the bulk of the actin is bundled and not found in the F-actin fraction.

[092] Fig. 8E is a photo of gel showing the bundling activity of SS15, SS2 and SS16 peptides *in vitro* after addition to unpolymerized actin with increased molar ratio of peptide:actin = 10:1 and 100:1. At a molar ratio of 10 and 100, the polymerised actin is predominantly in the bundled form, with only small amounts of free F-actin after the addition of SS15 and SS16. Notice in lane 6, where the SS12 active peptide was added at a molar ratio of 100:1, there is clearly no band of free F-actin and very small bands at the lower molar ratio of 10:1, showing that the most effective peptide in bundling all the actin is likely SS12. The SS15 peptide does not completely bundle all the actin until administered at a higher molar ratio than 10. The SS16 peptide however appears to exhibit a high activity in binding actin at a molar ratio of 10 and then reaches a maximum level of activity somewhere between a molar ratio of 10 and 100, after which the activity drops off dramatically.

[093] Referring now to Fig. 8F, the gel shows bundling activity of SS12 at molar ratio to actin of 10 and 50. Even at the upper levels of a molar ratio of 50, all of the actin is in the bundled form with no G-actin or free soluble F-actin.

[094] These experiments demonstrate that an *in vitro* dosage of a molar ratio of between 0.5 and 1 peptide to actin results in F-actin bundling. This represents the threshold dosage of peptide needed in order for the peptide to cause *in vitro* F-actin bundling.

#### EXAMPLE 9

##### Specificity of Synthetic Peptides

[095] Referring now to Fig. 7, since the SS2 sequence is specific to SuSy protein (with SS2 having some similarity to actin), we tested its effect on the well-known interaction of rabbit muscle aldolase with actin (Winter et al. 1998, Rabbit muscle aldolase was purchased from Sigma). Phalloidin could potentially interfere with the peptides of the invention since both drugs affect actin. However this gel shows that phalloidin does not have an affect on the peptide bundling activity and therefore it is possible to use both peptide and phalloidin in tandem for research applications.

[096] Addition of SS2 peptide had no effect on aldolase precipitation in the absence of actin (Fig. 7A), but increased the amount of aldolase co-precipitating with polymerized actin (Fig. 7B). Although binding of aldolase to actin is not sucrose dependent as shown for SuSy, the presence of Sucrose in the polymerization assay caused an additional increase in the amount of aldolase bound to polymerized actin as shown by lane 6 in Fig. 7B. Rabbit muscle aldolase (1 nmol) was incubated in the absence (lanes 1-3) or presence (lanes 4-6) of rabbit muscle actin (2 nmol). Addition of 100 nmol SS2 peptide (lanes 2, 3, 5 and 6) had no effect on aldolase solubility in the absence of actin (Fig. 7A), yet increased its coprecipitation with polymerized actin (lanes 3-6, Fig. 7B). Sucrose had an additional effect on aldolase: actin binding (lanes 3, 6).

#### EXAMPLE 10

##### *In Vivo* Administration of Active Synthetic Peptides

[097] Peptide Preparation and Treatment: Suspensions of SS12 peptide can be prepared by combining the peptide and PLURONIC F-68 block copolymer at 1:9 or 1:18 ratios of peptide to polymer, to prepare suspensions in the concentration range 0-10 mg/ml and 20 mg/ml respectively. The mixture is heated to between 150-190° C, and the peptide is solubilized in the

molten polymer to obtain a clear solution. This solution is then cooled to form a solid dispersion. The solid dispersion for intraperitoneal or intratumoral formulation can be hydrated with 2.5% dextrose solution (aqueous) by stirring at 4° C overnight to obtain a fine suspension.

[098] SS12 peptide is synthesized, weighed and dissolved in low salt buffer thorough mixing and sonication. Solubilizing agents can be added to the solution. Dilutions are made from this stock solution and the final excipient, 0.9% NaCl at 37° C, is added to each dose formulation just prior to dosing. The final ratio of liquid components (buffer, SS12, and saline) can be 5:5:90, respectively. Subjects having tumors are given an effective amount of the solution intratumorally, 0.1 to 0.5 ml, one to five times/week, using a syringe and a needle.

[099] After sufficient period of peptide administration, a noticeable decrease in the tumor cell growth and cell division should be observed. Administration of the active peptides should cause the bundling of F-actin in the tumor cells, thereby prohibiting the metastasis and growth characteristic of tumor cells.

[0100] The present examples, methods, procedures, treatments, specific compounds and molecules are meant to exemplify and illustrate the invention and should in no way be seen as limiting the scope of the invention. Any patents or publications mentioned in this specification are indicative of levels of those skilled in the art to which the patent pertains and are hereby incorporated by reference to the same extent as if each was specifically and individually incorporated by reference.

## PROVISIONAL CLAIMS

1. A peptide comprising a sequence selected from the following group:  
GIVRKWISRFEVWPYLKK (SEQ ID NO: 10); and  
GIVRKWISRFEVWPYLKL (SEQ ID NO: 12), wherein  
said peptide causes actin bundling and inhibits actin depolymerization.
2. A peptide of claim 1, having from 18 to 100 amino acids, wherein the amino acid sequence is at least 80% homologous with the native *Zea mays* protein sequence as set forth in GenBank Accession Number 1498382.
3. A method of stabilizing microfilaments by administering a therapeutically effective amount of the peptide of claim 1.
4. A method of treating cancer and preventing neoplastic cell growth by administering a therapeutically effective amount of the peptide of claim 1.
5. The method of claim 4, wherein the peptide of claim 1 is administered intratumorally.
6. A peptide useful in causing actin bundling, having from 7 to 100 amino acids, said peptide sequence having at least 90% homology to SEQ ID NO: 17 and, if extended beyond SEQ ID NO: 17, to the corresponding amino acid sequence of a native *Zea mays* sucrose synthase protein.
7. A method for inhibiting actin activity in a eukaryotic cell comprising administering to said cell a peptide having from 7 to 100 amino acids, said peptide sequence having at least 90% homology to SEQ ID NO: 17 and, if extended beyond SEQ ID NO: 1, to the corresponding amino acid sequence of a native *Zea mays* sucrose synthase protein.
8. A peptide having the sequence E H\* G I V\* R\* - W - - - - V\* W, where H\* means H or a conservative substitution therefore, V\* means V or a conservative substitution therefore, and R\* means R or a conservative substitution therefore, and - means any amino acid.

9. A peptide having the sequence E-x-G-[IV]-[IVL]-x(2)-W-x(5)-[IV]-W, where x means any amino acid and the number of amino acid residues is indicated, and the permissible conservative substitutions are indicated within the brackets.

## **ABSTRACT**

**[0101] Synthetic peptides derived from sucrose synthase useful for causing actin bundling and preventing actin depolymerization. These peptides can be useful for anti-cancer therapeutics, delivered to subjects solely, or concomitantly or sequentially with other known cancer therapeutics.**

# Fig. 1

SUS1	ENG <u>GILRKWIS</u> RFDVW	native
SUS2	ENG <u>IVRKWIS</u> RFEVW	native
Actin (consensus)	EHG <u>IVTNWDD</u> MEKIWHHTFY	
SS2	<u>GIVRKWI</u> SRFEVWPYLKK	active
SS11	ILRVPFRTENG <u>IVRKWI</u> <sub>(NH2)</sub>	inactive
SS12	<u>GIVRKWI</u> SRFEVWPYL <sub>(NH2)</sub>	active
SS15	<u>GIVRKAI</u> SRFEVAPYL <sub>(NH2)</sub>	less active
SS16	<u>SRFEVWPYL</u> <sub>(NH2)</sub>	less active
SP3	<sup>N</sup> RRISSE <sup>N N</sup> DKK <sub>(NH2)</sub>	inactive
NR11	GPTLKRTASTAFMNTTSKK	inactive
SP26	GRMRRIATVEMMKK	inactive
SS1	GDRVLSRLHSVRERIGK	inactive

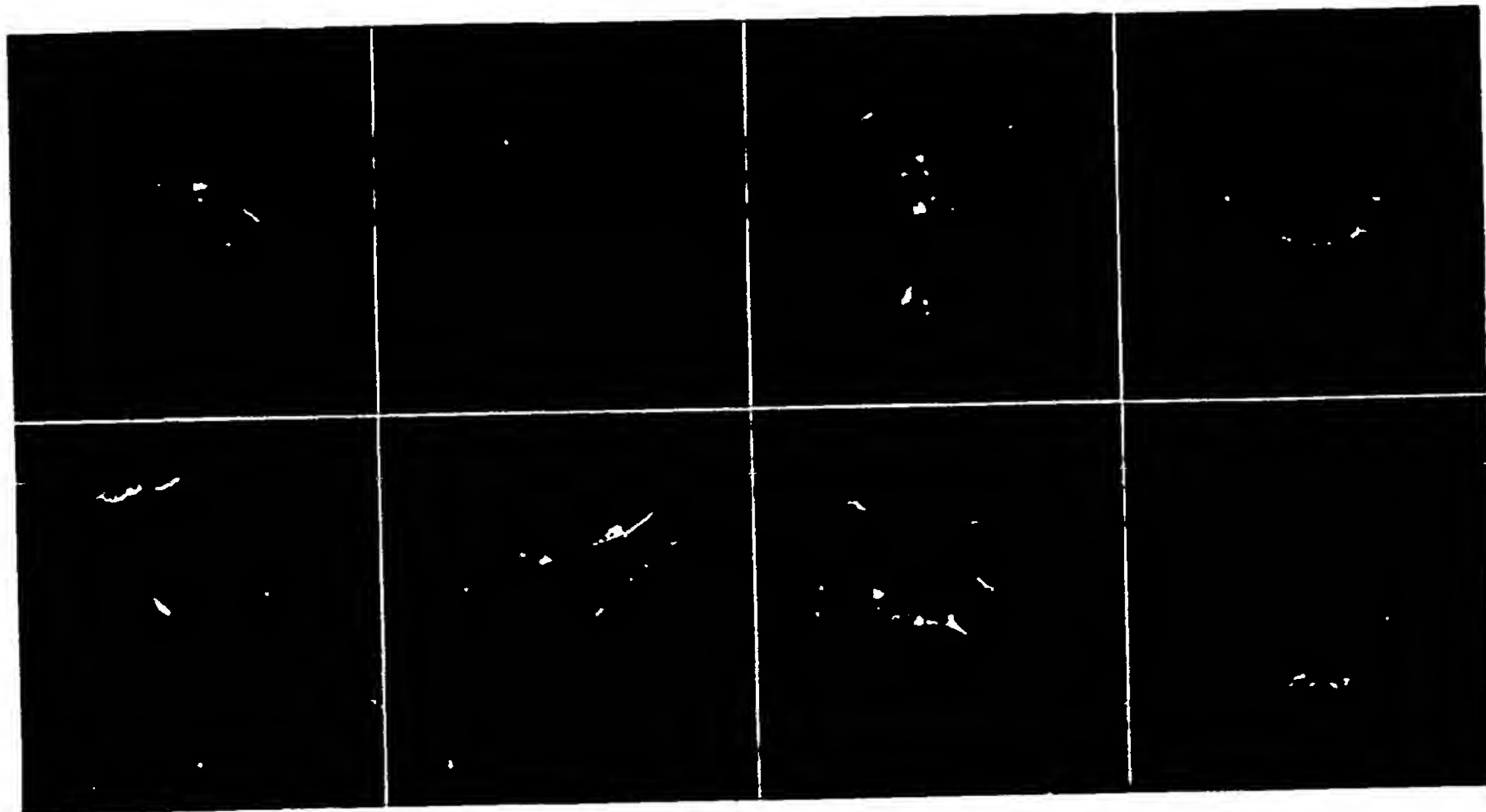
Double basic cluster: red

Possible region of specificity: green

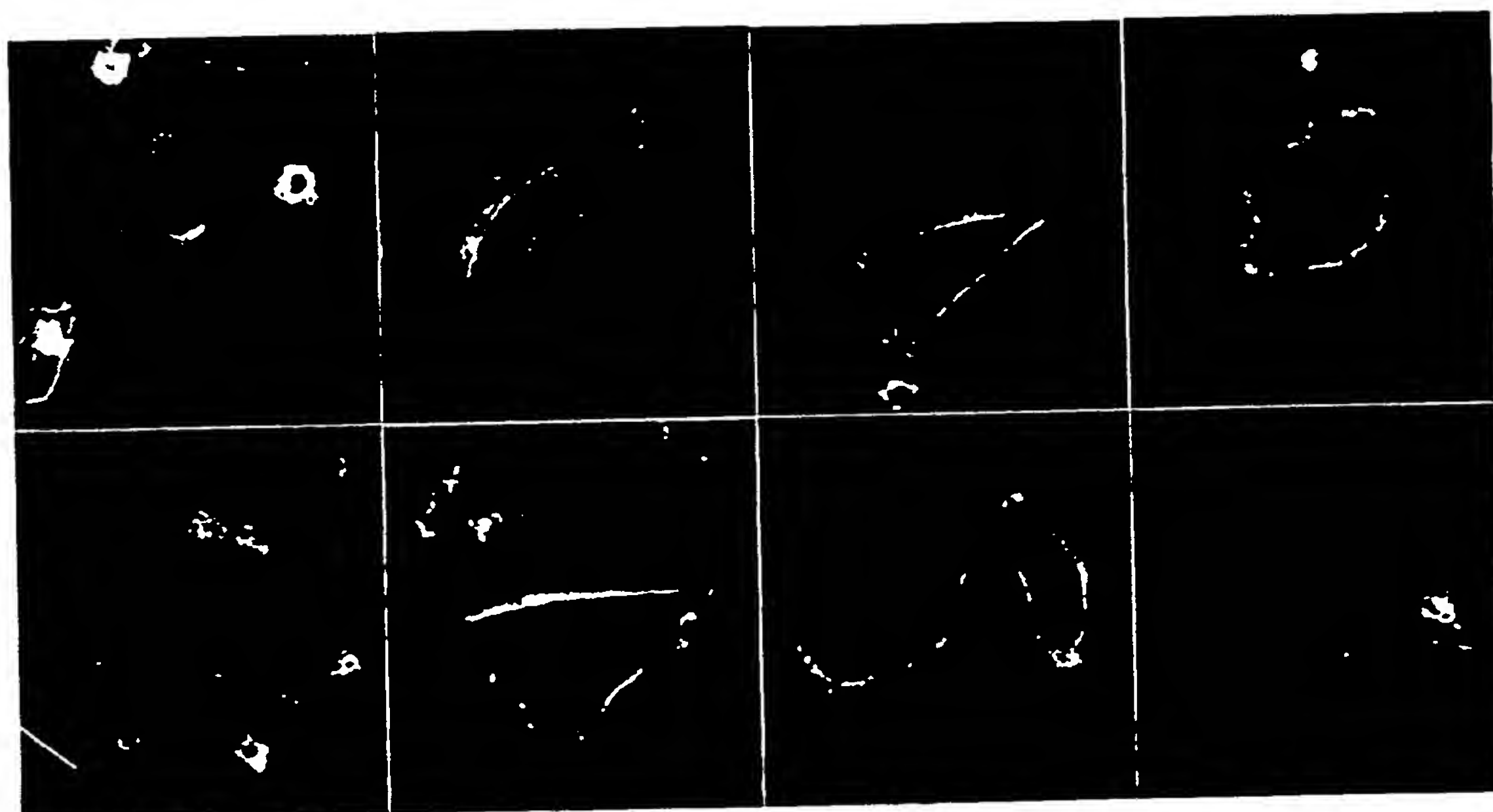
Substitutions: blue

Fig. 2

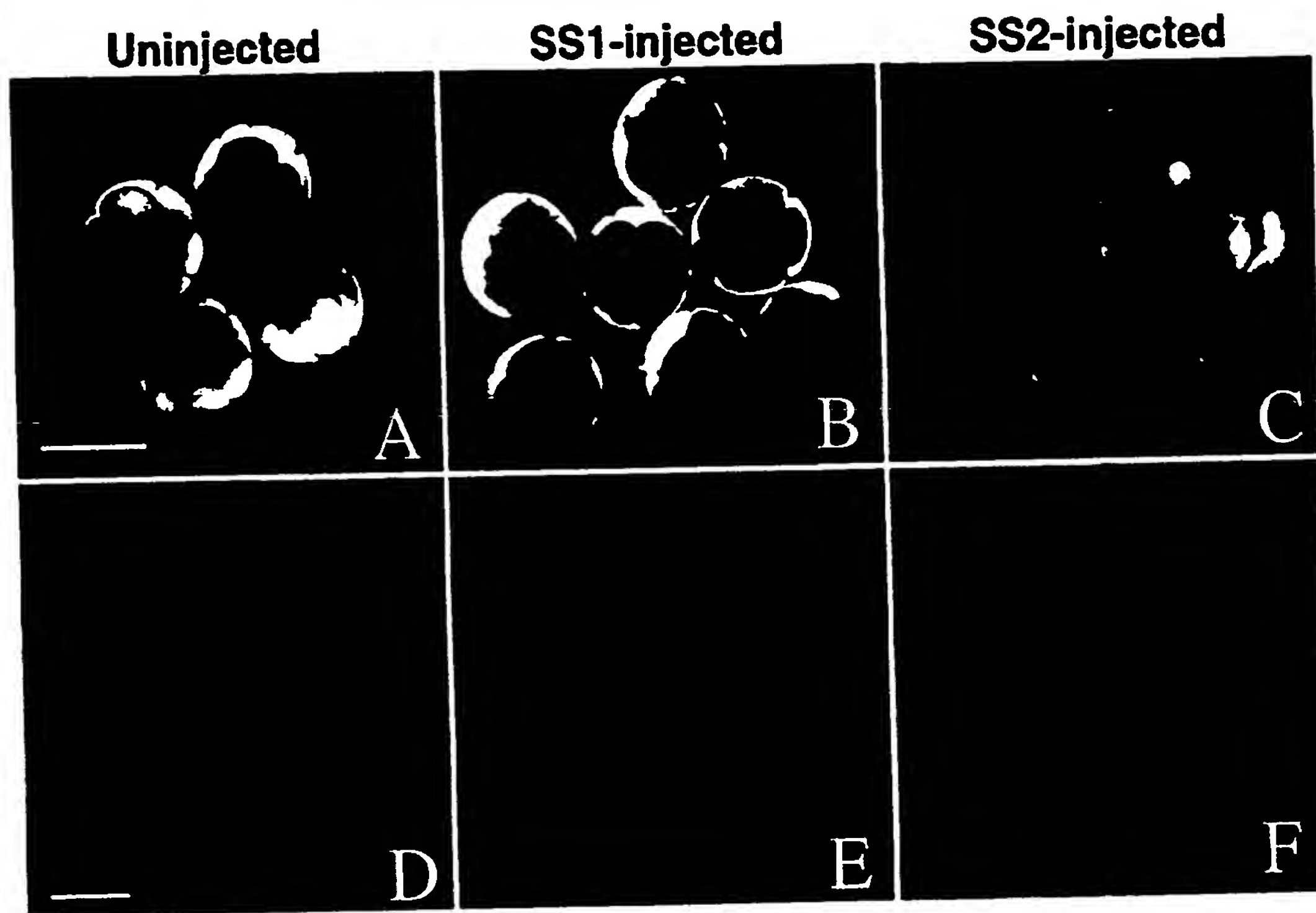
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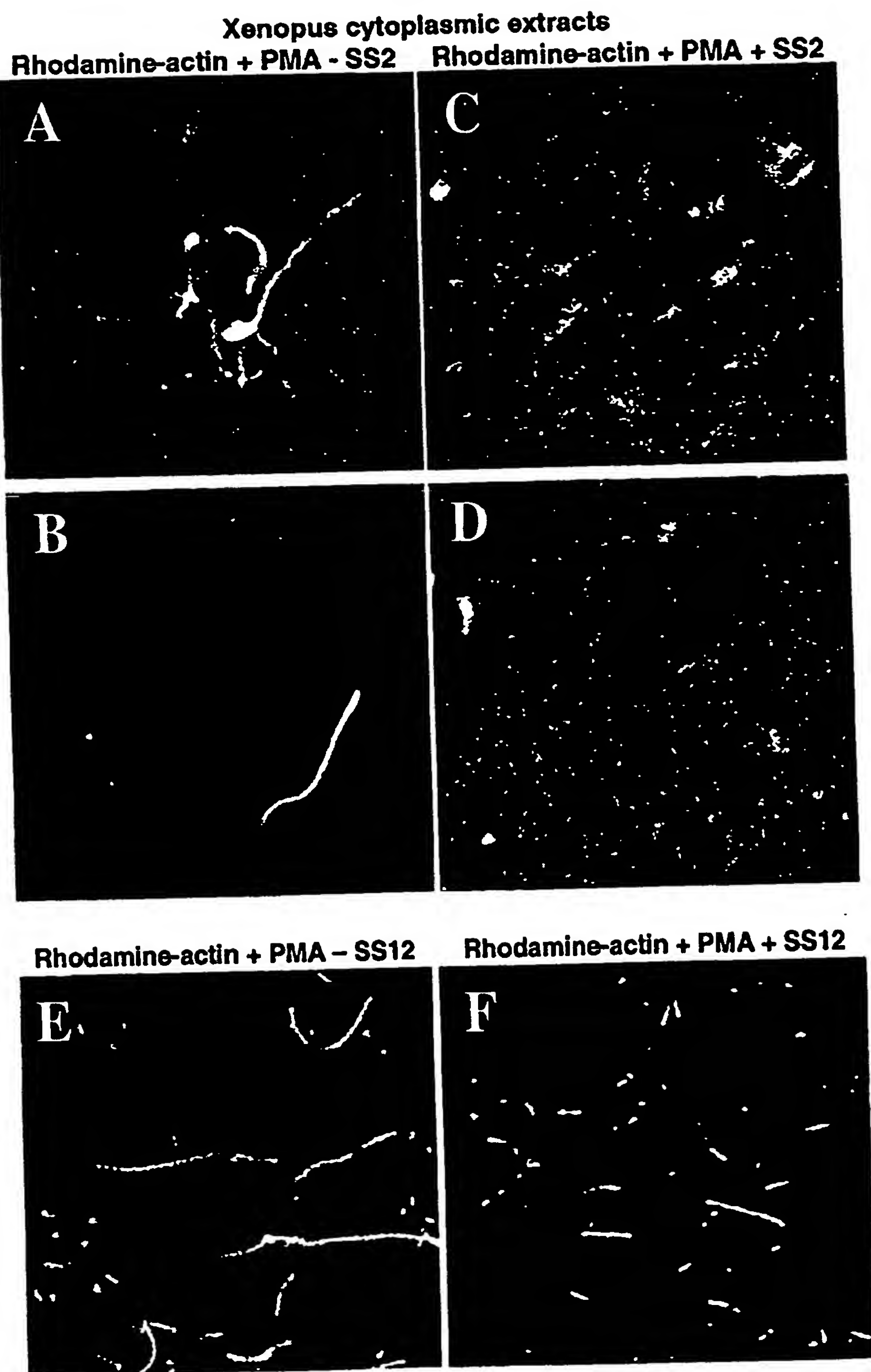
B



**Fig. 3**



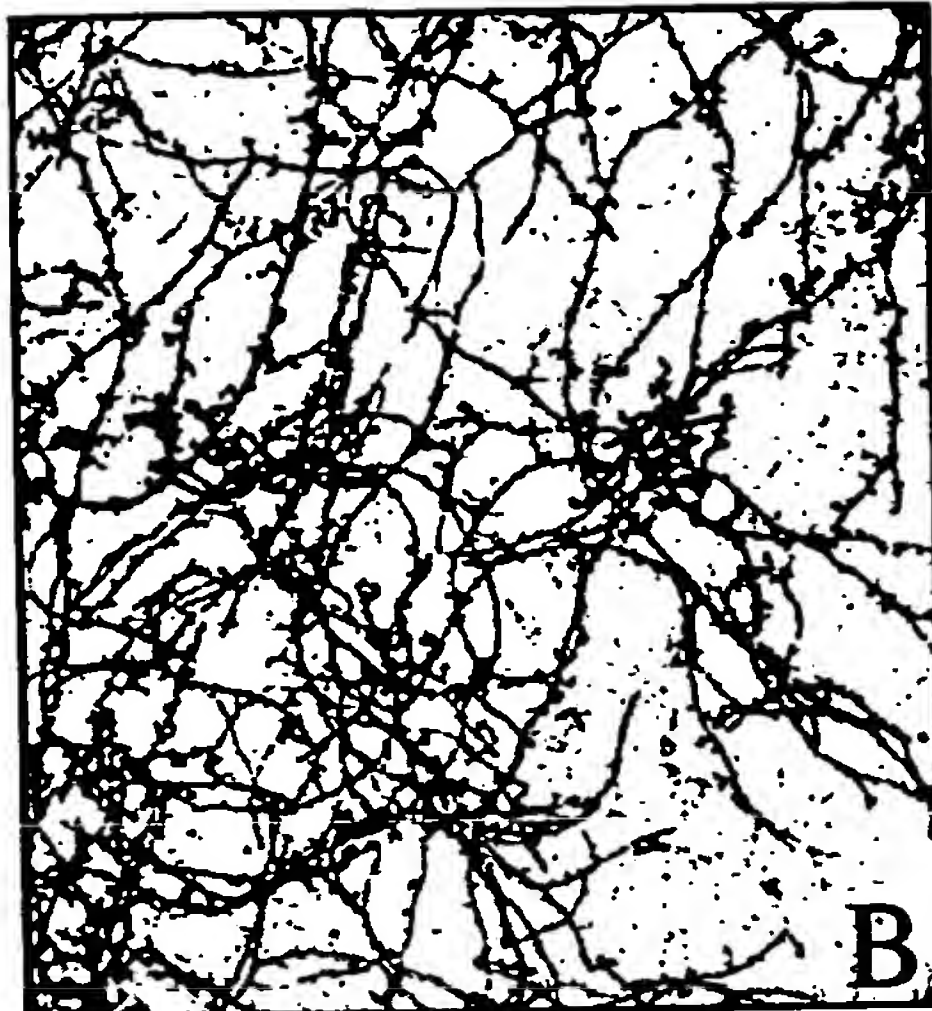
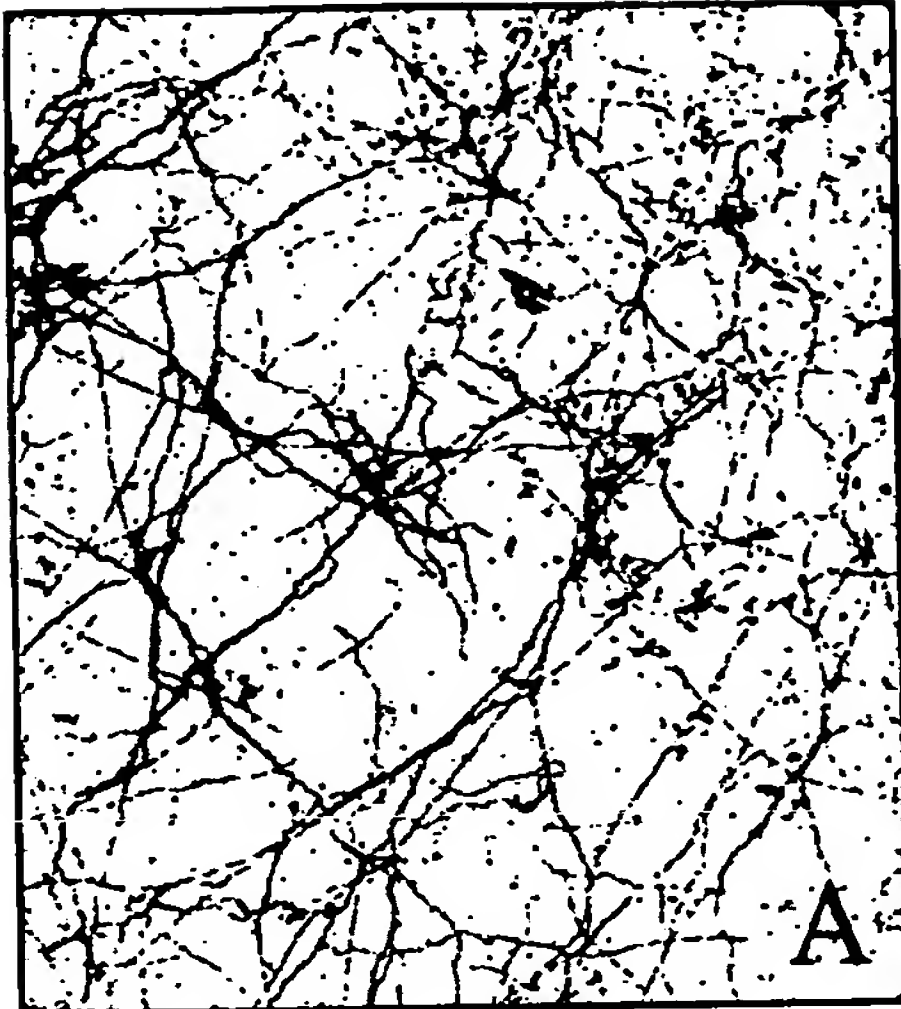
**Fig. 4**



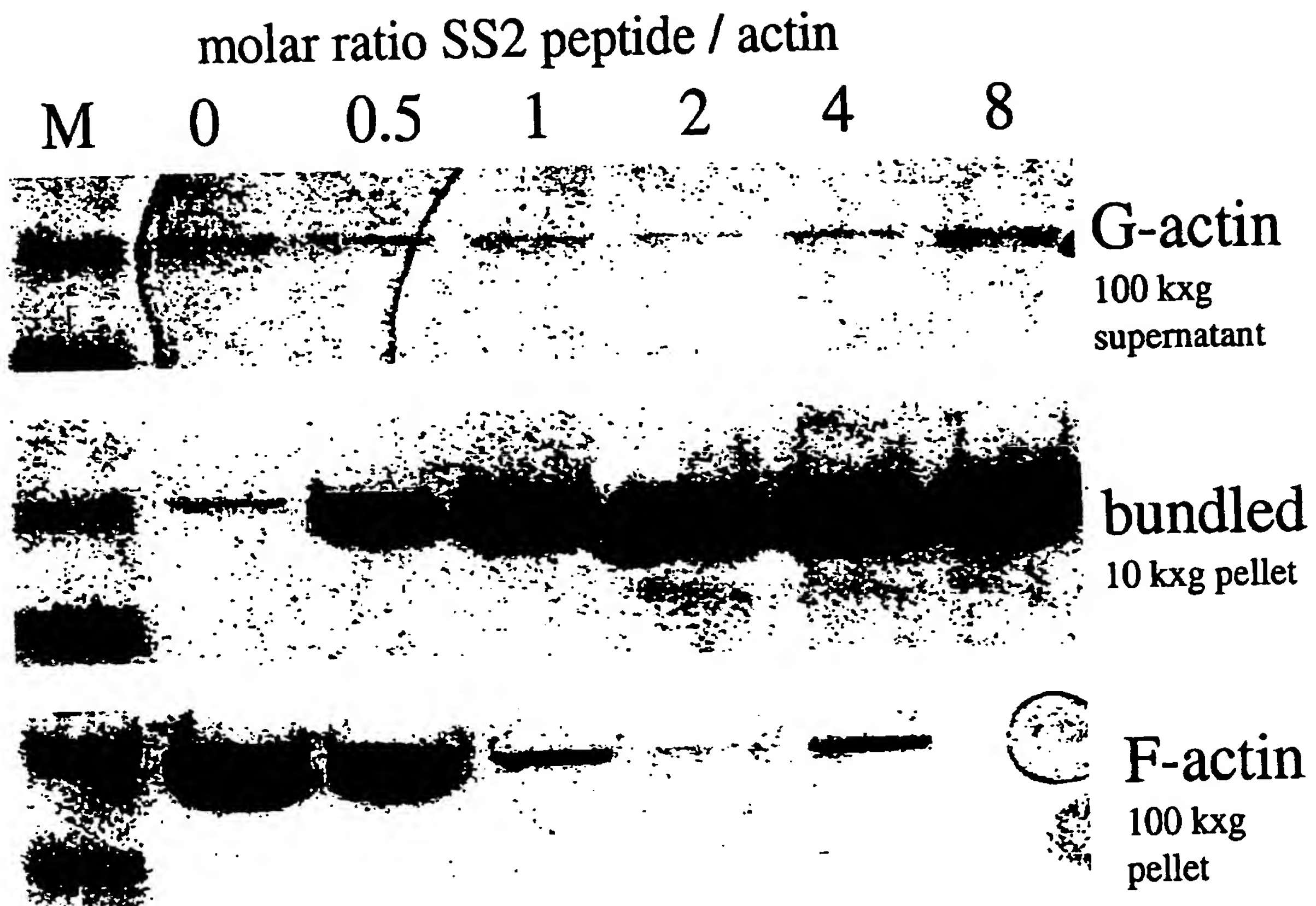
**Fig. 5**

control

SS12



**Fig. 6A**



**Fig. 6B**

**Effect of SS2 on polymerized  
F-actin in vitro**

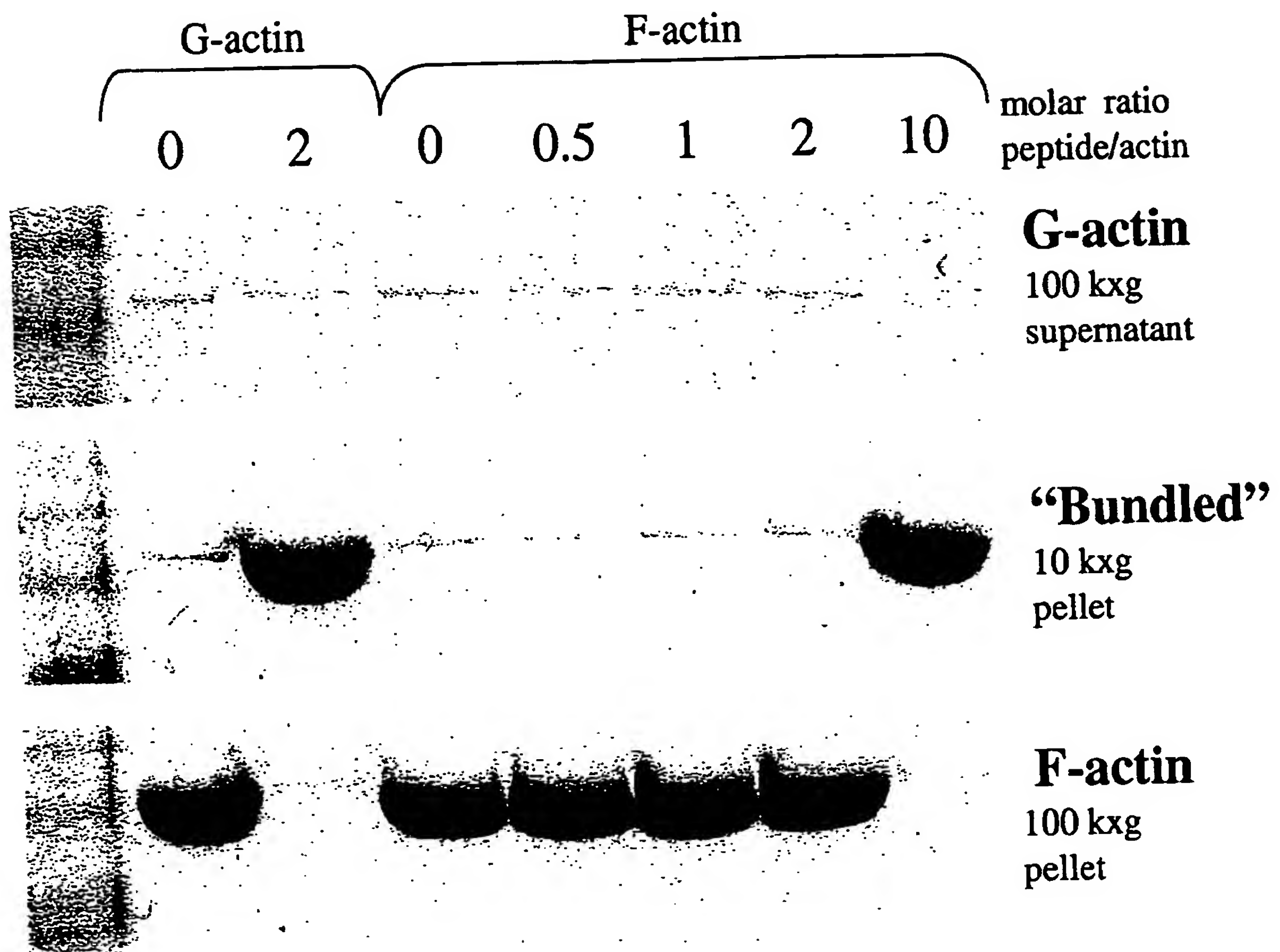
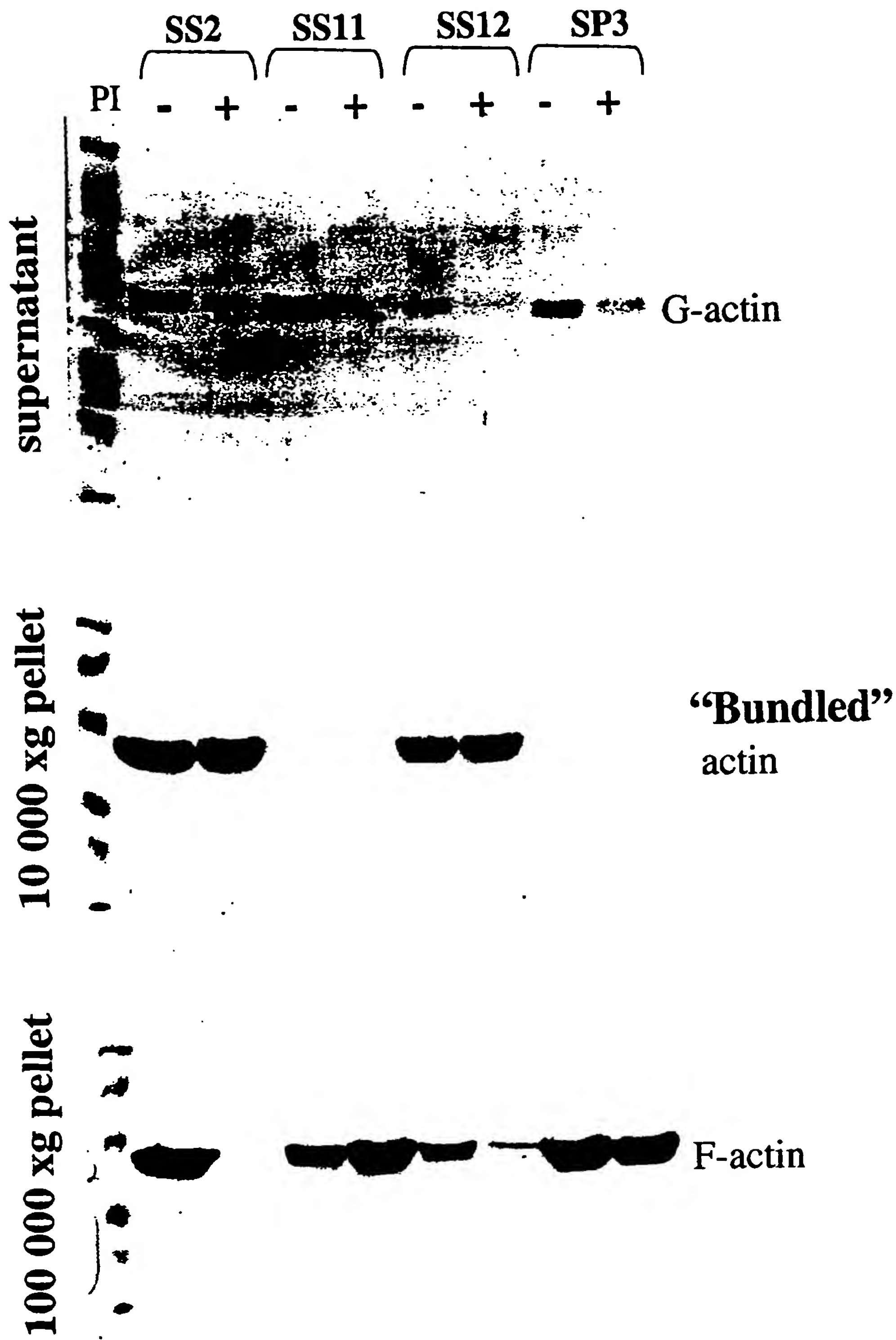
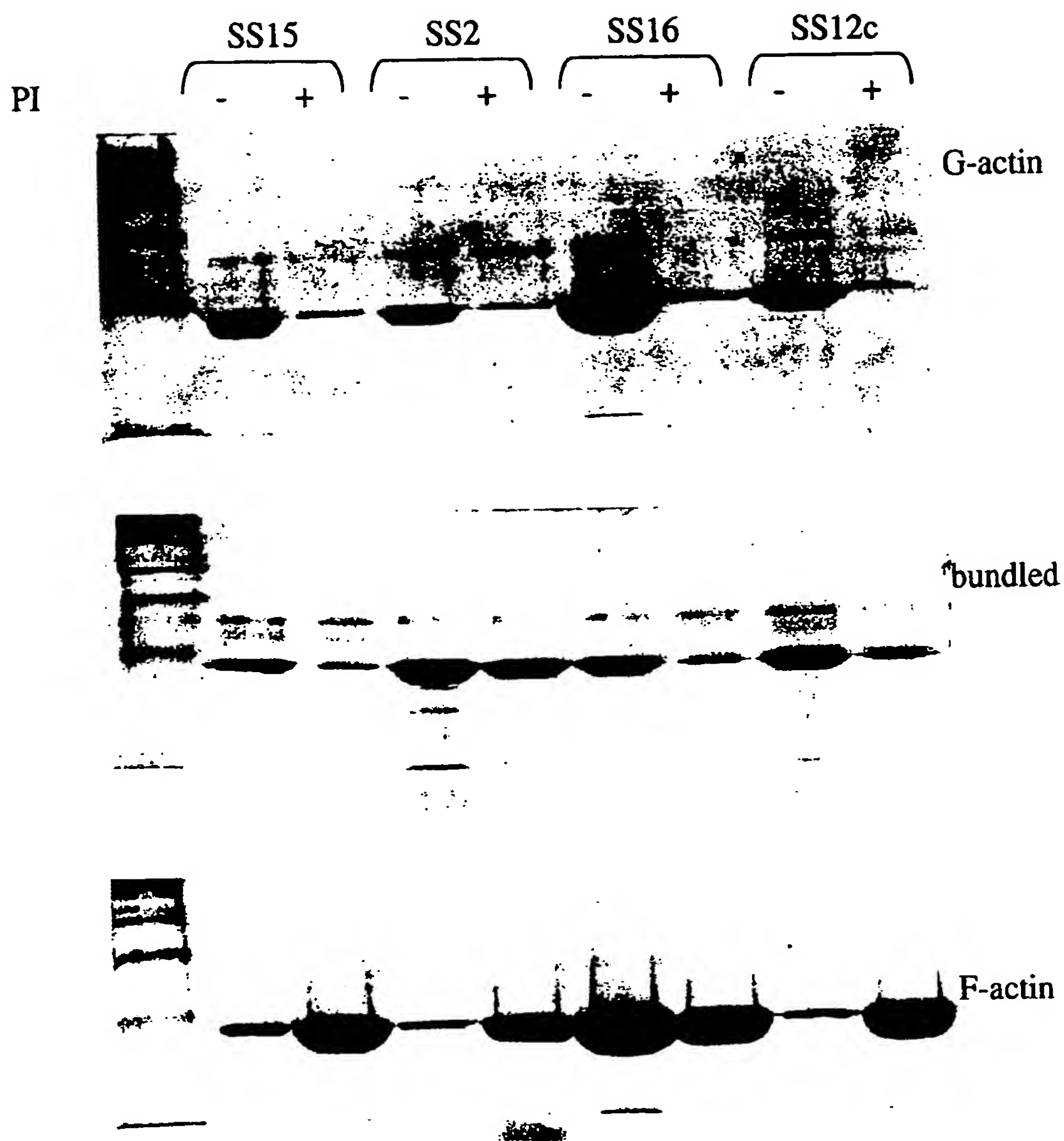


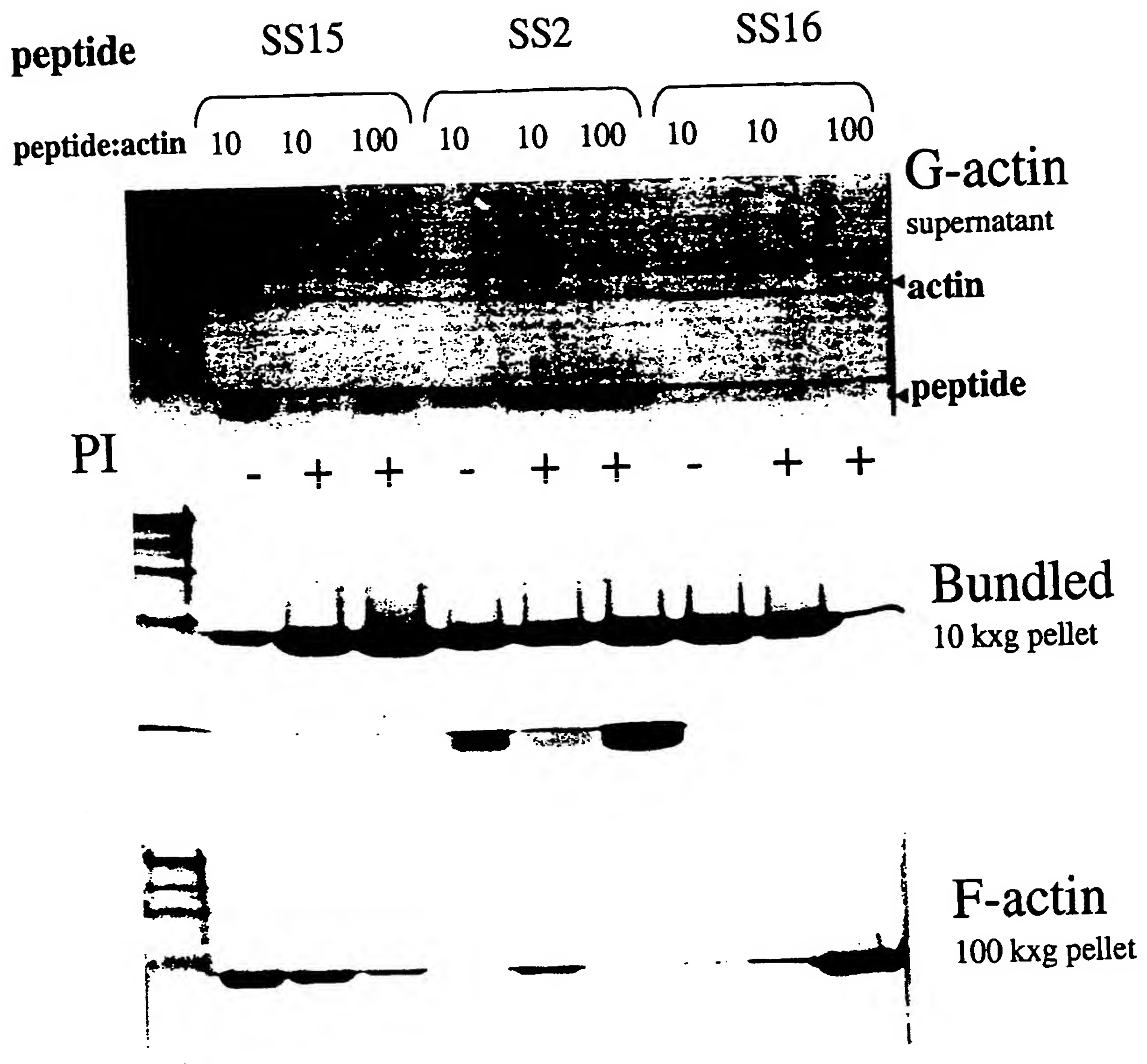
Fig. 6C



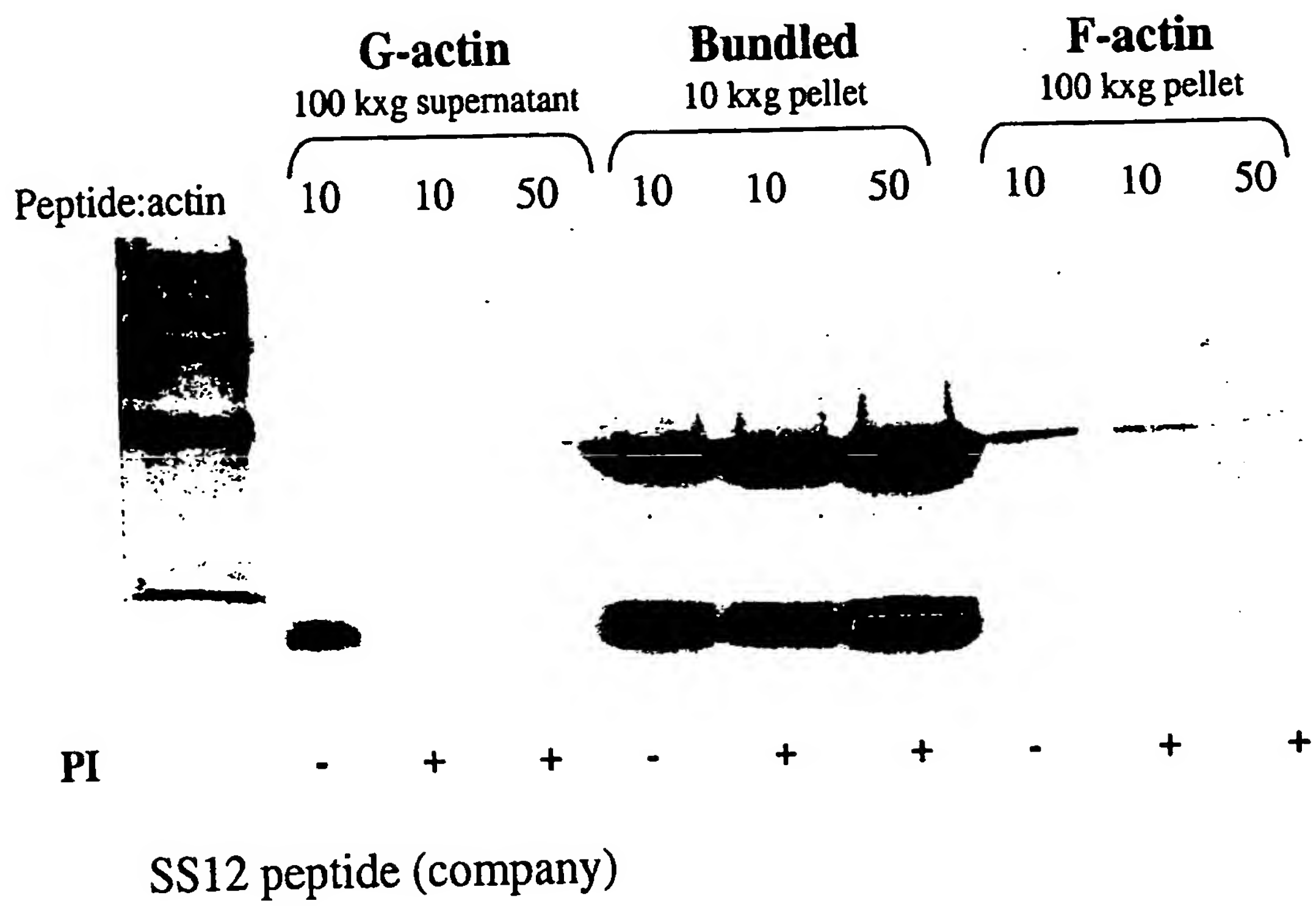
**Fig. 6D**



**Fig. 6E**

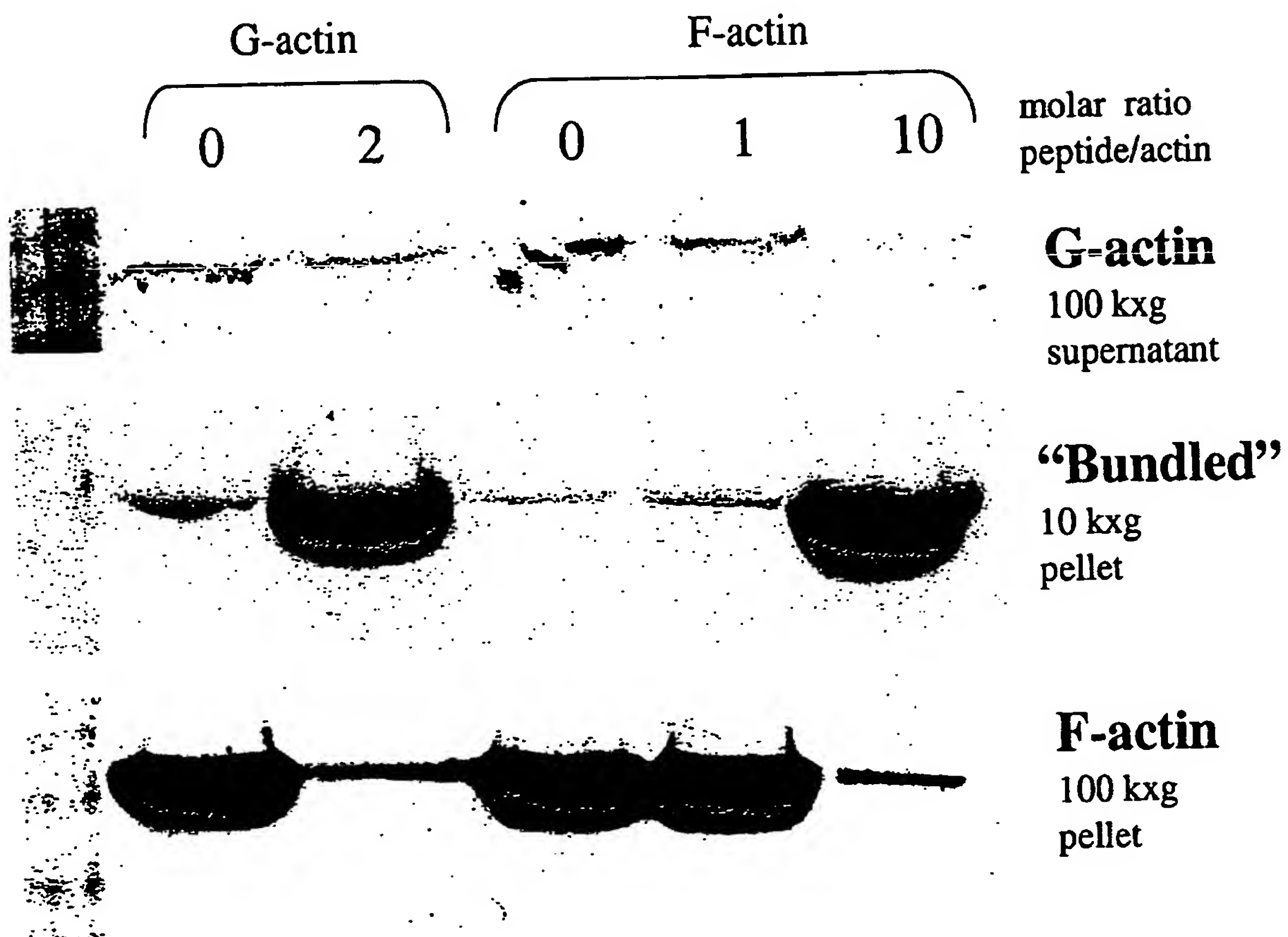


**Fig. 6F**



**Fig. 7**

SS2 bundling activity is not affected  
by phalloidin



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